



Consommation
et Corporations Canada

Consumer and
Corporate Affairs Canada

Bureau des brevets

Patent Office

Ottawa, Canada
K1A 0G9

(21) (A1)	2,067,754
(22)	1991/08/22
(43)	1992/02/25

5,076,5/84

(51) INTL.CL.³ A61K-009/127

(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Preparation for the Application of Agents in
Mini-Droplets

(72) Cevc, Gregor - Germany (Federal Republic of) ;

(73) Same as inventor

(30) (DE) P 40 26 833.0 1990/08/24
(DE) P 40 26 834.9 1990/08/24
(DE) P 41 07 153.0 1991/03/06
(DE) P 41 07 152.2 1991/03/06

(57) 30 Claims

Notice: The specification contained herein as filed

Canada

CCA 3254 (10-89) 41

Abstract

The invention relates to a preparation for the application of agents in the form of minuscule droplets of fluid, in particular provided with membrane-like structures consisting of one or several layers of amphiphilic molecules, or an amphiphilic carrier substance, in particular for transporting the agent into and through natural barriers such as skin and similar materials. The preparation contains a concentration of edge active substances which amounts to up to 99 mol-% of the agent concentration which is required for the induction of droplet solubilization. Such preparations are suitable, for example, for the non-invasive applications of antidiabetics, in particular of insulin. The invention, moreover, relates to the methods for the preparation of such formulations.

2067754

CEVC, Gregor

C 7041

International patent application

Preparation for the application of agents in mini-droplets

The present invention relates to a novel type of preparations suitable for the application of different agents in the form of a minuscule droplet or, in particular, a vesicle consisting of one or a few membrane-like amphiphile assemblies. These can mediate the transport of agents into and through a series of natural permeability barriers or through the constrictions in such barriers; for example, through intact skin or similar organs. The invention further relates to a procedure for the large-scale production of such carriers. As a special example, non-invasive application of antidiabetics is described for the case of insulin.

The application of various agents is often hampered by the presence of barriers with a low permeability to such agents. Owing to skin impermeability, for example, many common therapeutic agents must be applied per os or parenterally (i.v., i.m., i.p.). Intrapulmonary and intranasal applications of aerosols, the use of rectal formulations, gels for mucous applications, or use of ocular formulations are only practicable in certain areas and not for all types of drugs. The transport of different agents into plant tissues is subject to even more severe constraints due to the high permeability barrier of the cuticular wax layers.

Noninvasive drug application through permeability barriers thus would be advantageous in many cases. In humans and animals one would expect such a percutaneous application of agents to protect the agents from degradation in the gastrointestinal tract; modified drug distribution could possibly also be achieved. Such drug application, moreover, would influence the pharmacokinetics of the agent molecules and permit simple as well as multiple noninvasive therapy. (Karzel K., Liedtke, R.K. (1989) *Arzneim. Forsch./Drug Res.* 39, 1487-1491). In the case of plants, improved penetration

into or through the cuticle could reduce the drug concentration required for a given application and thus significantly diminish pollution problems (Price, C.E. (1981) In: The plant cuticle (D.F. Cutler, K.L. Alvin, C.E. Price, Edits.), Academic, New York, pp. 237-252).

There are many reports on different attempts to increase the permeability of intact skin by suitable manipulations (cf. Karzel und Liedtke, op. cit.). Jet injection (Siddiqui & Chien (1987) Crit. Rev. Ther. Drug. Carrier. Syst. 3, 195-208.), the use of electric fields (Burnette & Ongpipattanakul (1987) J. Pharm. Sci. 76, 765-773) or chemical penetration enhancers, such as solvents and surfactants, are particularly worth mentioning. A long list of additives which have been used to enhance the penetration of one particular water soluble agent (Nolaxon) into skin, for example, is given in the work by Aungst et al. (1986, Int. J. Pharm. 33, 225-234). This list encompasses nonionic substances (including long-chain alcohols, surfactants, zwitterionic phospholipids, etc.), anionics (most notably fatty acids), cationic long-chain amines, sulfoxides as well as different amino-derivatives; amphotheric glycines and betaines are also mentioned. Despite all this, the problem of agent penetration into skin has as yet not at all - or not satisfactorily - been solved.

A survey of procedures used for increasing the penetration of agents through a plant cuticle is given in the work by Price (1981, op.cit.). To date it has been common to simply add chemical penetration enhancers to the mixture of agent and other molecules; applications to human skin were the only case in which additives were sometimes applied in advance, in the form of an organic solution. The reason for this application form was the current concept for the action of penetration enhancers: to date one has studied, discussed, and believed

2067754

- 3 -

that, in general, any facilitated agent penetration is a consequence of skin fluidization, on the one hand (Golden et al., (1987) J. Pharm. Sci. 76, 25-28). (This phenomenon is normally associated with a destruction of the skin surface and of its protective shield and thus is undesired.) On the other hand, it has been shown that some agents can permeate through skin in the form of low-molecular weight complexes with added molecules (Green et al., (1988) Int. J. Pharm. 48, 103-111).

Methods deviating from the ones already described have brought little improvement to date. The use of lipoidal carriers, the liposomes, on intact skin, which has been theoretically discussed by several authors, was mainly aimed at modifying the agent's pharmacokinetics (Patel, Bioch. Soc. Trans., 609th Meeting, 13, 513-517, 1985, Mezei, M. Top. Pharm. Sci. (Proc. 45th Int. Congr. Pharm. Sci.F.I.P.) 345-58 Elsevier, Amsterdam, 1985). Thus far, all proposal of this kind, moreover, involved the use of standard lipid vesicles (liposomes) which cannot penetrate the skin at all or permeate through the skin very inefficiently, as is shown in this patent application. Patent applications nos. JP 61/271204 A2 [86/271204] refer to a related use of liposomes in which hydrochinonglucosidal is employed to improve the stability of the agent.

Hitherto available preparations for percutaneous use have mostly been applied under occlusion; in the case of liposomal preparations, this was even a general rule. The corresponding preparations only contained small or lipophilic substances, as well as a limited number of skin-fluidizing additives. Correspondingly, they afforded only partial control over the pharmacokinetic properties of final preparations. In an attempt to improve this situation a proposal was made (WO 87/1938 A1) to use drug-carrying lipid vesicles in combination with a gelatinizing agent as a transdermal patch. This has

prolonged drug action but has not increased the skin-penetration capability of the drug itself. Through massive use of penetration enhancers (polyethylene glycol and fatty acids) and of lipid vesicles, Gesztes und Mezei (1988, Anesth. Analg. 67, 1079-1081) have succeeded in inducing local analgesia with lidocaine-containing carriers; however, the overall effectiveness of the drug in this preparation was relatively low and its effects were only observed several hours after the beginning of an occlusive application.

By a specially designed formulation we have succeeded in obtaining results which were dramatically better than those of Gesztes and Mezei. Our carrier formulations consisted of filtered lipid vesicles (liposomes) which also contained some detergents, with a declared optimum lipid/surfactant content of 1-40/1, in practice mainly around 4/1.

These results provided a basis for German patent application P 40 26 834.9-41 which also refers to German patent application P 40 26 833.0-43; the latter deals with the problem of liposome fabrication.

Since then, we have unexpectedly discovered that certain criteria, described in this application, may be formulated for the qualification of drug carriers as suitable for the penetration into and through a permeability barrier. The main requirement of such a drug carrier - which in the following is called a transfersome - is that it is sufficiently elastic to penetrate through the constrictions in a barrier, such as skin. In the case of transfersomes consisting of phosphatidylcholine and sodium cholate this condition is fulfilled when the edge tension of a carrier is below 10 Piconewton; similar values are also likely to pertain to other, related systems. Carriers which are capable of creating a gradient after an application are particularly

206775₄

- 5 -

useful; this is due to the fact that they have a spontaneous tendency for penetration through permeability barriers.

It is, therefore, an object of the present invention to specify the properties of novel preparations which are suitable for the mediation of rapid transport of diverse agents and other substances through permeability barriers and constrictions.

A further object of this invention is to introduce a new class of carrier preparations for the transport of drugs through human, animal or plant skin, which result in a characteristic improved availability of the agent molecules at the target site.

It is yet another object of this invention to prepare formulations for non-invasive application of antidiabetics, most notably of insulin; these should ensure an improved, therapeutically sufficient, and reproducible form of drug application.

A further object of this invention is to provide procedures for the production of such preparations.

These objects have been accomplished through the features of the independent claims.

Advantageous embodiments are mentioned in the subclaims.

The transfersomes according to this invention differ from the liposomes hitherto described for topical application and from other related carriers in at least three basic features. Firstly, they can consist of an arbitrary amphiphile, including oils. Secondly, they can be made in arbitrary fashion: their penetration capacity does not depend on the

2067754

- 6 -

manufacturing procedure. Thirdly, the penetration capability of the previously described liposomes optimized for applications on skin (cf. patent application P 40 26 834.9-41) was based on the use of a carrier composition with an optimal lipid/surfactant ratio in the range of $L/S=1-40/1$. However, a transfersome must mainly have an optimal elasticity, which ensures a sufficiently high permeation capability of such a carrier. If this basic requirement is fulfilled by the addition of edge-active substances to a basic transfersome component, the necessary total amount of the edge-active substance can correspond to L/S values below $1/500$ (in the case of classical surfactants below $1/50$ to $1/100$). The range of concentrations suitable for making transfersomes is thus by several thousand per cent higher than previously believed.

Transfersomes also differ from micellar carrier formulations in at least two basic features. Firstly, a transfersome is, as a rule, far bigger than a micelle; consequently, it also obeys different diffusion laws. Secondly, and more importantly, a transfersome typically contains a water-filled central core (the inner lumen of a vesicle). Nearly all water soluble substances can be incorporated in the core of a transfersome and thus transported across a permeability barrier. Transfersomes are suitable for transporting amphiphilic and lipophilic substances.

If simple carriers are not sufficiently deformable and their permeation capacity must be achieved by using certain edge-active additives, the concentration of the latter is then preferably in the range between 0.1 and 99 % of the quantity which would be required for carrier solubilization. Frequently, the optimum - depending on the purpose and the drug used - is located in the range between 1 and 80 %, most frequently between 10 and 60 % of the solubilization dose; the

concentration range between 20 and 50 mol-% is the most preferred dose.

Our novel transfersomes can mediate transport of agents through essentially all permeability barriers and are suitable, for example, for percutaneous (dermal) applications of medical agents. Transfersomes can carry water- or fat-soluble agents to various depths at the application site, depending on the transfersomal composition, application dose, and form. Special properties which cause a carrier to behave as a transfersome can be realized for phospholipid vesicles as well as for other types of amphiphile aggregates.

In this application it is shown for the first time that by means of suitably formulated transfersomes, a major proportion of the drugs applied can be introduced not only into a permeability barrier, such as skin, but, moreover, can be transported into the deeper tissues where they become systemically active. Transfersomes can carry polypeptides, for example, through intact skin at an effectiveness which is a 1,000 times higher than was previously possible when using structureless penetration enhancers. Transfersomally formulated substances can reach nearly 100 % of the corresponding biological or therapeutical maximum efficacy after applications on human skin. Similar effects, to date, have only been achievable by using an injection needle.

In the course of this study, it has surprisingly been found that through use of such novel drug carriers, antidiabetics can be brought into the blood through intact skin without the necessity of auxiliary measures such as an injection. After a dermal application of insulin applied in the form of transfersomes, more than 50 % and often more than 90 % of the applied drug dose are routinely found in the destined organs of the body. Insulin-containing, dermally applied

transfersomes can thus successfully replace injections of insulin solutions.

The present invention, consequently, opens up a way for simple, noninvasive and completely painless therapy of type II diabetes: transfersomes can be used alone or in combination with an arbitrary dosing means for non-problematic therapy of acute and/or chronic diabetes.

Carriers according to this invention can consist of one or several components. Most commonly, a mixture of basic substances, one or several edge-active substances and agents is used. Lipids and other amphiphiles are best suited basic substances; surfactants or suitable solvents are the best choice from the point of view of edge-active substances. All these can be mixed with agents in certain proportions depending both on the choice of the starting substances and on their absolute concentrations. It is possible that one or several preparation components are only made edge-active by subsequent chemical or biochemical modification of a preparation (ex tempore and/or in situ).

Transfersomes thus offer an elegant, uniform and generally useful means of transport across permeability barriers for diverse agents. These newly developed carriers are perfectly suited for use in human and animal medicine, dermatology, cosmetics, biology, biotechnology, agrotechnology and other fields.

A transfersome according to this invention comprises any carrier with a special capability to get or diffuse into or through a permeability barrier under the effect of a gradient and by so doing to transport material between the application and destination sites.

2067754

- 9 -

A (drug) carrier of this type preferably corresponds to a molecular homo- or hetero-aggregate or to a polymer. The carrier aggregate, according to this invention, consists of a few or many, identical or different molecules; these form a physico-chemical, physical, thermodynamical and, quite frequently, functional unity. Some examples of corresponding aggregates are micelles, disk-micelles, oil-droplets (nanoemulsions), nanoparticles, vesicles or 'particulate emulsions'; parts of an aggregate can also be held together by (a) non-covalent force(s). The optimal carrier size is also a function of the barrier properties. Furthermore, it is influenced by the polarity (hydrophilicity), mobility (dynamics), and charge density as well as the elasticity of an carrier (surface). Advantageous sizes of transfersomes are in the range of 10 nm to 10,000 nm.

For dermal applications, for example, preferably particles or vesicles with a diameter of the order of 100-10,000 nm, frequently in the range of 100 to 400 nm, and most frequently with sizes between 100 and 200 nm are used as carriers.

For the use in plants, relatively small carriers, depending on the details of each individual application, should be used, most frequently with diameters below 500 nm.

DEFINITIONS

LIPIDS

A lipid in the sense of this invention is any substance with characteristics similar to those of fats or fatty materials. As a rule, molecules of this type possess an extended apolar

region (chain, X) and, in the majority of cases, also a water-soluble, polar, hydrophilic group, the so-called head-group (Y). The basic structural formula 1 for such substances reads



where n is greater or equal zero. Lipids with n=0 are called apolar lipids; those with n ≥ 1 are polar lipids. In this context, all amphiphiles, such as glycerides, glycerophospholipids, glycerophosphinolipids, glycerophosphonolipids, sulfolipids, sphingolipids, isoprenoidlipids, steroids, sterines or sterols and lipids containing carbohydrate residues, can simply be referred to as lipids.

A phospholipid, for example, is any compound of formula 2



In this formula, n and R₄ have the same significance as in formula 1 except that R₁ and R₂ cannot be hydrogen, an OH-group or a short chain alkyl residue; R₃ is a hydrogen atom or an OH-group, in the majority of cases. In addition, R₄ can be a short chain alkyl group substituted by three short chain alkylammonium residues, e.g. trimethylammonium, or an amino-substituted short chain alkyl, e.g. 3-trimethylammonioethyl (cholinyl).

A lipid is preferably any substance according to formula 2, in which n=1, R₁ and R₂ is hydroxyacyl, R₃ is a hydrogen atom and R₄ is a 2-trimethylammonioethyl (the last compound corresponding to the phosphatidylcholine headgroup), 2-dimethylammonioethyl, 2-methylammonioethyl or 2-aminoethyl (corresponding to

a phosphatidylethanolamine headgroup).

A lipid of this kind is, for example, phosphatidylcholine from natural sources, in the old nomenclature also called lecithin. This can be obtained, for example, from eggs (then being rich in arachidic acid), soy-bean (rich in C-18 chains), coconuts (rich in saturated chains), olives (rich in monounsaturated chains), saffron, safflower and sunflowers (rich in n-6 linolenic acid), linseed (rich in n-3 linolenic acid), from whale-oil (rich in monounsaturated n-3 chains), from Nachtkerze or primrose (rich in n-3 chains), etc. Preferred natural phosphatidylethanolamines (in the old nomenclature also called cephalins), frequently stem from egg or soy-beans.

Further preferred lipids are synthetic phosphatidylcholines (R_4 in formula 2 corresponding to 2-trimethylammonioethyl), synthetic phosphatidylethanolamines (R_4 being identical to 2-aminoethyl), synthetic phosphatidic acids (R_4 being a proton) or their esters (R_4 corresponding e.g. to a short chain alkyl, such as methyl or ethyl), synthetic phosphatidylserines (R_4 corresponding to an L- or D-serine), or synthetic phosphatidyl(poly)alcohols, such as phosphatidylglycerol (R_4 being identical to L- or D-glycerol). In this case, R_1 and R_2 are identical acyloxy residues such as lauroyl, oleoyl, linoyl, linoleoyl or arachinoyl, e.g. dilauroyl-, dimyristoyl-, dipalmitoyl-, distearoyl-, diarachinoyl-, dioleoyl-, dilinoyl-, dilinoleoyl-, or diarachinoylphosphatidylcholine or -ethanolamine, or different acyl residues, e.g. R_1 = palmitoyl and R_4 = oleoyl, e.g. 1-palmitoyl-2-oleoyl-3-glycerophosphocholine; or different hydroxyacyl residues, e.g. R_1 = hydroxypalmitoyl and R_4 = hydroxyoleoyl; or mixtures thereof, e.g. R_1 = hydroxypalmitoyl and R_4 = oleoyl etc. R_1 can also signify an alkenyl and R_2 identical hydroxyalkyl residues, such as tetradecylhydroxy or hexadecylhydroxy, e.g.

in ditetradecyl- or dihexadecylphosphatidylcholine or -ethanolamine, R_1 can be an alkenyl and R_2 a hydroxyacyl, e.g. a plasmalogen (R_4 = trimethylammonioethyl), or R_1 can be an acyl, e.g. myristoyl, or palmitoyl, and R_2 a hydroxy, e.g. in natural or synthetic lysophosphatidylcholines or lysophosphatidylglycerols or lysophosphatidylethanolamines, e.g. 1-myristoyl- or 1-palmitoyllysophosphatidylcholine or -phosphatidylethanolamine; R_3 is frequently hydrogen.

A convenient lipid according to this invention is also a lipid of the basic formula 2, in which $n=1$, R_1 is an alkenyl residue, R_2 is an acylamido residue, R_3 is a hydrogen atom and R_4 is 2-trimethylammonioethyl (choline residue). A lipid of this kind is known under the term sphingomyeline.

Furthermore, suitable lipids are analogs of lysophosphatidylcholine, such as 1-lauroyl-1,3-propandiol-3-phosphorylcholine, monoglycerides, such as monoolein or monomyristin, a cerebroside, a ganglioside or a glyceride which contain no free or esterified phosphoryl- or phosphono group or a phosphino group in the position 3. One example of such glyceride is diacylglyceride or 1-alkenyl-1-hydroxy-2-acylglyceride with arbitrary acyl or alkenyl groups, the 3-hydroxy group in these then being ether-bonded to one of the mentioned carbohydrate residues, such as a galactosyl residue, for example in monogalactosylglycerol.

Lipids with desired head or chain group properties can also be prepared biochemically, using e.g. phospholipases (such as phospholipase A1, A2, B, C, and especially D), desaturases, elongases, acyl-transferases, etc., starting with any natural or synthetic precursor.

Suitable lipids, furthermore, are all lipids found in

biological membranes and extractable with suitable apolar organic solvents, such as chloroform. In addition to the lipids already mentioned, this group of lipids also encompasses steroids, such as oestradiols, or sterines, such as cholesterol, beta-sitosterine, desmosterine, 7-keto-cholesterol or beta-cholestanol, fat-soluble vitamins, such as retinoids, vitamins, such as vitamin A1 or A2, vitamin E, vitamin K, such as vitamin K1 or K2, or vitamin D1 or D3, etc.

EDGE ACTIVE SUBSTANCES

An edge active substance according to this application is any substance which is capable of inducing or increasing the carrier system's capacity to form edges, protrusions or relatively strongly curved surfaces; this property also manifests itself in the capability to induce pores in lipid structures, such as membranes, or even provoke a solubilization (lysis) in the higher concentrations ranges. More strictly speaking, all such substances are considered edge-active which exhibit a tendency to accumulate at or near the edges between the polar and apolar parts of molecules and/or near or at the edges between the polar and apolar parts of the supramolecular aggregates, thereby lowering the free energy for the formation of edges and/or strongly curved surfaces. All surfactants and many solvents as well as asymmetric, and thus amphiphatic, molecules or polymers, such as many oligo- and polycarbohydrates, oligo- and polypeptides, oligo- and polynucleotides or their derivatives also belong to this category.

The edge activity of the used 'solvents', surfactants, lipids, or agents depends on the effective relative hydrophilicity or hydrophobicity of each molecule, and can also be modified by the choice of further system components and boundary conditions in the system (temperature, salt content, pH value,

etc.). Functional groups, such as double bonds in the hydrophobic part of molecules, which lower the hydrophobicity of this molecular region, increase edge activity; elongation or space-demanding substituents in the hydrophobic molecular parts, e.g. in the aromatic part, lower the edge activity of a substance. Charged or strongly polar groups in the headgroup normally increase the edge activity provided that the hydrophobic molecular part has remained the same. Direct connections between the lipophilic and/or amphiphilic system components have the reverse effect.

Solvents which are to some extent edge active only in certain concentration ranges encompass simple, especially short chain, alcohols, such as methanol, ethanol, n-propanol, 2-propen-1-ol (allyl alcohol), n-butanol, 2-buten-1-ol, n-pentanol (amyl alcohol), n-hexanol, n-heptanol, n-octanol and n-decanol; furthermore, iso-propanol, iso-butanol or iso-pentanol. Higher alcohols are even more potent, for example, ethandiol (ethylene glycol), 1,2-propane diol (propylene glycol), 1,3-propane diol, 1,3-butane diol, 2,3-butane diol, propane triol (glycerol), 2-butene-1,4-diol, 1,2,4-butane triol, 1,3,4-butane triol, 1,2,3-butane triol, butane tetraol (erythritol), 2,2-bis(hydroxymethyl)1,3-propane diol (pentaerythritol), 2,4-pentadiol and other pentadiols or pentendiols, 1,2,5-pentantriol and other pentantriols or pententriols, pentantetraol, 1,2,6-hexane triol and other hexane triols, hexane tetraol and -pentaol, heptane diol, - triol, -tetraol, -pentaol and -hexaol, 1,4-butane diol- diglycidyl-ether, etc. Short-chain, di-, tri-, tetra-, penta- and hexaoxyethylene glycols and -ethylene glycols are also suitable for the present purpose as well as cyclic alcohols, such as benzyl alcohol, cyclopentanol, cyclohexanol, 3-, 4-, 5-cyclohexanol, cyclohexyl alcohol, aryl-alcohols, such as phenyl-ethanol, etc.

Edge active solvents which can be used according to this invention include, furthermore, short-chain acyl-, alkyl-, alkenyl, hydroxyacyl-, alkenyloxy- as well as aryl derivatives of different acids and bases, such as acetic acid, formic acid, propionic acid, butenoic acid, pentenoic acid, etc. of many amino acids, benzoic acid, phosphoric- and sulphuric acid, of ammonia, purine, pyrimidine, etc., provided that they do not impair the chemical integrity of the carriers and the agent molecules to an unacceptable extent.

A nonionic edge active substance is any material which contains at least one, and in the majority of cases several, strongly hydrophilic groups and at least one, sometimes also several relatively hydrophobic, water insoluble residues. 'Nonionic' edge active substances can be zwitterionic or truly non-ionic.

Free of any charge and edge active are e.g. the lipoidal substances of the basic formula 3

$$R_1 - ((X_i - Y_j)_k - Z_l)_m - R_2 \quad (3)$$

in which X, Y and Z are different polar (hydrophilic) or apolar (hydrophobic) groups, which confer an amphiphatic character to the whole molecule. Z is mainly a water soluble residue and i, j, k, l and m are greater or equal zero. R_1 and R_2 are two arbitrary residues; the first is mostly polar or very short; the second apolar.

The residues R_2 or X in such lipids often represent an acyl-, alkyl-, alkenyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain with 8-24 carbon atoms. Very frequently, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, n-dodecyl, n-tetradecyl or n-tetradecenoyl, n-hexadecyl, n-

hexadecenoyl, n-octadecyl, n-octadecenoyl and n-octadecendienyl, n-octadecentrienyl, etc. are used.

Sorbitol is one possible example of residue Z. $(X_i - Y_j)$ can be a polyene, polyoxyalkane, such as polyoxyethylene, polyalcohol, such as polyglycol, or polyether. $(X_i - Y_j)$ mainly contain 1-20 and very frequently 2-10 units, e.g. in ethylene glycol, di- and triglycol (oligoglycol) or polyethylene glycol.

In simple substances according to formula 3, the residue R_1 or R_2 is frequently an alkyl-, alkenyl-, hydroxyalkyl-, alkenyl-hydroxy- or hydroxyacyl-chain with 1-24 carbon atoms. Very suitable are substances such as n-dodecyl (lauryl-ether), n-tetradecyl (myristoyl-ether), n-pentadecyl (cetyl-ether), n-hexadecyl (palmitoyl-ether), n-octadecyl (stearoyl-ether), n-tetradecenoyl (myristoleoyl-ether), n-hexadecenoyl (palmitoleoyl-ether) or n-octadecenoyl (oleoyl-ether). Owing to their good availability, the following substances are, amongst others, frequently used: 4-lauryl-ether (Brij 30), 9-lauryl-ether, 10-lauryl-ether, 23-lauryl-ether (Brij 35), 2-cetyl-ether (Brij 52), 10-cetyl-ether (Brij 56), 20-cetyl-ether (Brij 58), 2-stearyl-ether (Brij 72), 10-stearyl-ether (Brij 76), 20-stearyl-ether (Brij 78), 21-stearyl-ether (Brij 721), 2-oleoyl-ether (Brij 92), 10-oleoyl-ether (Brij 96) and 20-oleoyl-ether (Brij 78), the increasing number in their names indicating an increasing headgroup length. Suitable substances of this class are marketed under the names GENAPOL, THESIT and LUBROL.

Amongst the most common nonionic surfactants of the ether-type which are suitable for the present purpose are the substances of the Myrj trademark, such as polyoxyethylene(8)-stearate (Myrj45), polyoxyethylene(20)-stearate (Myrj49), polyoxy-

ethylene(30)-stearate (Myrj51), polyoxyethylene(40)-stearate (Myrj52), polyoxyethylene(50)-stearate (Myrj53), polyoxyethylene(100)-stearate (Myrj59), etc. Further products of these classes are sold under the trademark Cirrasol ALN; common polyoxyethylene-alkylamides are e.g. surfactants of the trademark Atplus.

Another important special form of the nonionic edge active substance according to basic formula 3 most frequently contains a hydroxyl group in the position of residue R_1 and a hydrogen atom in the position of residue R_2 , by and large. Residues X and Z are frequently an alkoxy- or alkenoxy-, in principle also a hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain with 4-100 carbon atoms. Residue Y, too, is frequently an alkoxy-, alkenoxy-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain but one which is often branched and carries one methyl- or ethyl-side chain. Perhaps the most widely used edge active substances of this class are the surfactants which are marketed under the trademark "Pluronic".

Further, very commonly used special forms of non-ionic edge active substances are sold under the trademark "TWEEN". The cyclic part of this substance class is frequently a sorbitol ring. Residues R_1 , R_2 , R_3 and R_4 are frequently of the alkoxy- or alkenoxy-, and even more commonly of the polyene-, polyoxyalkene-, such as polyoxyethylene-, polyalcohol-, such as polyglycol-, or polyether type. Some of these chains can be apolar, corresponding to e.g. an acyl-, alkyl-, alkenyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain with 8-24 carbon atoms. If none of residues R_1 , R_2 , R_3 or R_4 is apolar, one of the side-chains of a branched chain or one of the termini must be hydrophobic.

Chains in the substances of TWEEN type are very frequently of

the polyoxyethylene class. They mainly contain one terminal hydrogen atom and more rarely a methoxy group. One of the polyoxyethylene chains, however, contains a hydrophobic residue which preferably corresponds to an acyl-, alkyl-, alkenyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-chain with 4-24, and in particular 12-18 carbon atoms.

Edge active substances which are sold under the trademark "TRITON" are also useful according to this invention.

Polyalcohol residues R_2 are most frequently esterified or etherified; however, in some cases they can also be bound to the hydrophobic chain through a nitrogen atom. They are very often adducts of ethyleneglycol, glycerol, erythritol, or pentaerythritol, for example 1-alkyl-, 1-alkenyl-, 1-hydroxyalkene-glycerol, or corresponding 1,2-, or 1,3-diglycerides (for example, 1-alkyl,2-alkyl-, 1-alkyl,2-alkenyl-, 1-alkenyl,2-alkyl-, 1-alkenyl,2-alkenyl-, 1-alkenyl,2-hydroxyalkyl-, 1-hydroxyalkyl,2-alkenyl-, 1-alkyl,2-hydroxyalkyl-, 1-hydroxyalkyl,2-alkyl-, 1-alkenyl,2-hydroxyalkene-, 1-hydroxyalkene,3-alkenyl-, 1-alkyl,3-alkyl-, 1-alkyl,3-alkenyl-, 1-alkenyl,3-alkyl-, 1-alkenyl,3-alkenyl-, 1-alkenyl,3-hydroxyalkyl-, 1-hydroxyalkyl,3-alkenyl-, 1-alkyl,3-hydroxyalkyl-, 1-hydroxyalkyl,3-alkyl-, 1-alkenyl,3-hydroxyalkene- or 1-hydroxyalkene,3-alkenyl-). Glycerol can be replaced by another oligo- or polyalcohol, such as erythritol, pentantriol, hexantriol, -tetraol or -pentaol, etc., resulting in a wide variety of linkage possibilities.

Z or R_2 , moreover, can contain one or more 1-10, preferably 1-6, most frequently 1-3 carbohydrate residues or their derivatives. 'Carbohydrate residue' in this context has the meaning as already described and is an alpha or beta and L- or D-alloside, -altroside, -fucoside, -furanoside, -galactoside,

-galactopyranoside, -glucoside, -glucopyranoside, -lactopyranoside, -mannoside, -mannopyranoside, -psicoside, sorboside, -tagatoside, -taloside; frequently used derivatives of disaccharides are L- or D-maltopyranoside, -maltoside, -lactoside, malto- or -lactobionamide; the corresponding derivatives of maltotriose or -tetraose are also useful.

The carbohydrate residue can also contain a sulfur atom, e.g. in beta-L- or D-thioglucopyranoside or -thioglycoside.

zwitterionic surfactants are substances, for example, which contain a sulphonate group, such as (3-((3-cholamidopropyl)-dimethylyammonio)-1-propanesulfonate (CHAPS) and (3-((3-cholamidopropyl)-dimethylyammonio)-2-hydroxy-1-propane-sulfonate (CHAPSO) or N-octyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (lauryl-sulfobetaine), N-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (myristyl-sulfobetaine), N-hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (palmityl-sulfobetaine), N-octadecyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate (stearyl-sulfobetaine), 'N-octadecenoyl-N,N,-dime-thyl-3-ammonio-1-propanesulfonate (oleoyl-sulfobetaine) etc.

Zwitterionic surfactants are also substances with the basic formula 4



in which n is one or zero. One of both side chains R_1 and R_2 contains one acyl-, alkyl-, alkenyl-, alkenoyl-, hydroxyalkyl-, hydroxyalkenyl- or hydroxyacyl-, or alkoxy chain with 8-24 carbon atoms each; the other residue corresponds to a hydrogen, to a hydroxy group or to a short chain alkyl

residue. R_3 normally represents a hydrogen atom or a short alkyl chain. X is most frequently anionic, e.g. in a phosphate- or sulfate-residue. The residue R_4 in this case is cationic, in order to ensure that the whole molecule is zwitterionic. Most frequently, ammonio-alkyl derivatives, such as ethanol-, propanol-, butanol-, pentanolamine, hexanolamine, heptanolamine or octanolamine, N -methyl-, N,N -dimethyl, or N,N,N -trimethyl-ammonio-alkyl, N -ethyl-, N,N -diethyl, or N,N,N -triethyl-amino-alkyl, unequal N -alkyles, such as N,N -methyl-ethyl-ammonio-alkyl, or corresponding hydroxyalkyl substances are used, sometimes in a substituted form. (Single chain (lyso) derivatives of all biological zwitterionic phospholipids as well as their modified forms (such as Platelet-Activating-Factor and its analogs) also belong to this category.) R_4 can also be a positively charged carbohydrate residue, such as an aminosugar or one of its derivatives. R_4 and X , moreover, can exchange positions.

An ionic edge active substance is any material which contains at least one positive or negative charge and at least one segment which is poorly water soluble. An anionic substance of this kind can also contain several charges but must have a negative total charge. The total charge of any cationic substance must be positive.

Anionic edge active substances are for example the substances described by the basic formula 5:



in which R_1 is an organic hydrocarbon residue, which can also be substituted, and G^{+} is a monovalent counterion, chiefly an alkali metal cation (such as lithium, sodium, potassium,

rubidium, or cesium), an ammonium ion or a low weight tetraalkylammonium-ion, such as tetramethylammonium or tetraethylammonium.

The hydrocarbon residue R_1 in an anionic surfactant of the basic formula 5 is frequently a straight chain or branched acyl, alkyl or alkenoyl, or oxidized or hydroxygenated derivative thereof; the residue R_1 can also contain one or several cyclic segments.

R_1 chain frequently contains 6-24, more frequently 10-20, and most frequently 12-18 carbon atoms; if unsaturated, it contains 1-6, and even more frequently 1-3, double bonds in n-3- or n-6- position.

The following hydroxyalkyl chains are preferred for the present purpose: n-dodecylhydroxy (hydroxylauryl), n-tetradecylhydroxy (hydroxymyristyl), n-hexadecylhydroxy (hydroxycetyl), n-octadecylhydroxy (hydroxystearyl), n-eicosylhydroxy or n-docosylhydroxy. Amongst the hydroxyacyl chains, the hydroxylauroyl, hydroxymyristoyl, hydroxypalmitoyl, hydroxystearoyl, eicosylhydroxy or docosylhydroxy chains are especially worth mentioning; particularly interesting amongst the hydroxyalkene-residues are the hydroxydodecen, hydroxytetradecen, hydroxyhexadecen, hydroxyoctadecen, hydroxyeicosen, hydroxydocosen, most notably 9-cis,12-hydroxy-octadecenyl (ricinolenyl) or 9-trans,12-hydroxy-octadecenyl (ricinelaidyl), 5-cis,8-cis,11-cis,14-cis,15-hydroxy-eicosatetraenyl (15-hydroxy-arachidonyl), 5-cis,8-cis,11-cis,14-cis,15-hydroxy,17-cis-eicosapentaenyl, 4-cis,7-cis,10-cis,13-cis,15-hydroxy,16-cis-docosapentaenyl and 4-cis,7-cis,10-cis,13-cis,15-hydroxy,16-cis,19-cis-docosahexaenyl.

Another class of anionic, edge active substances corresponds

to basic formula 6



here, R_1 is a hydrocarbon residue which can also be substituted; X is a short-chain alkyl residue and Y denotes a sulfonate-, sulfate-, phosphate-, phosphonate or phosphinate group. G^+ is a mostly monovalent counterion (cation).

Alkali metal alkyl- or -alkenylethersulfonates or -phosphates belong to this class of ether-bonded molecules. Special examples are sodium- or potassium-n-dodecyloxyethylsulfate, -n-tetradecyloxyethylsulfate, -n-hexadecyl-oxyethylsulfate or -n-octadecyloxyethylsulfate or an alkali metal alkane sulfonate, such as sodium- or potassium-n-hexanesulfonate, n-octansulfonate, n-decansulfonate, n-dodecansulfonate, -n-tetradecansulfonate, -n-hexadecansulfonate or -n-octadecansulfonate.

The substances of general formula 7



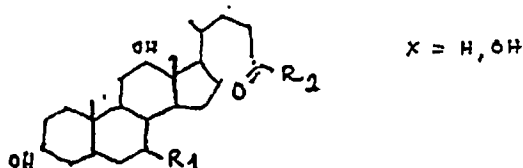
are related to the compounds of basic type 6. These are analogous to the substances of formula 6 but contain a directly (covalently) coupled charged headgroup.

Particularly useful anionic, edge active substances of above formula 6 are alkali metal-alkylsulfates. To mention just a few examples: sodium or potassium-n-dodecyl (lauryl)-sulfate, -n-tetradecyl (myristyl)-sulfate, -n-hexadecyl (palmityl)-sulfate, -n-octadecyl (stearyl)-sulfate, n-hexadecylen (palmitolein)-sulfate and n-octadecylen (olein)-sulfate. Instead of a sulfate group, sulfonate, n-methyl- or n-ethylglycine for example can also be used.

Various salts of bis-(2-alkyl-alkyl)-sulfosuccinate are also suitable for the applications as described in this work. Preferably, these are used as lithium-, sodium-, potassium-, or tetramethylammonium-bis-(2-ethyl-hexyl)-sulfosuccinate.

Furthermore, sarcosides, as well as alkyl- or alkenoyl-sulfochloride derivatives of the protein condensates, sulfonamide soaps, sulfatated or phosphorylated alcohol-esters, sulfatated or phosphorylated amides or monoglycerides, moreover, fatty acid alkylamides, sulfo- or phospho-succinic acid esters, taurides, alkylphenol-, alkylbenzol-, alkyl-napthaline-ethersulfonates etc., are also all useful.

Another important group of anionic edge active substances are the derivatives of cholic acid. Their basic formula reads



here, R_1 corresponds to a proton, an OH - or a carbonyl group and R_2 can be a derivative of taurine or glycocoll, for example. Particularly suitable are various salts of cholic acid (bile acid, 3 α ,7 α ,12 α -trihydroxy-5 β -cholane-24-oin-acid), deoxycholic acid (3 α ,12 α -dihydroxy-5 β -cholane-24-oin-acid), chenodeoxycholic acid, glycocholic acid (N-(3 α ,7 α ,12 α -trihydroxy-24-oxycholane-24-yl-)glycine), deoxycholic acid, glycodeoxycholic acid (N-(3 α ,12 α -dihydroxy-24-oxycholane-24-yl-)glycine), glycochenodeoxycholic acid, glycolitocholic acid, glycoursodeoxycholic acid, lithocholic acid, taurodeoxycholic

acid, taurocholic acid (3 α ,7 α ,12 α -trihydroxy-5 β -cholan-24-oin-acid-N-(sulfoethyl)amide), taurochenodeoxycholic acid, tauroglycocholic acid, taurolithocholic acid, taurolithocholic acid-3-sulfate, tauroursodeoxycholic acid, ursocholanic acid, ursodeoxycholic acid (3 α ,7 β -dihydroxy-5 β -cholanic acid), the most common counterions being sodium or potassium.

Diverse cholic acid esters, such as cholesteryl-alkyl-, -alkenyl-, -hydroxyalkyl-, -hydroxyalkene-esters or cholesterylsulfates and -sulfonates are also edge active according to this invention.

Related synthetic adducts of the CHAPS class can also be used; in this case, R₂ is frequently an NH-(CH₂)₃-N',N'-(CH₂)₂(CH₂)₂-R₃-CH₂-SO₃ segment, whilst R₃ can be a proton or a carbonyl group. Again, sodium or potassium are the most commonly used counterions.

Digitonines as well as saponines, such as Quillaja acid, have similar basic structures in their cores as the cholic acid derivatives; consequently, they can also be used as edge active substances according to this invention.

The basic formula of the phosphorus-containing anionic edge active substances is



in which n is zero or one. One of the two side chains R₁ and R₂ contains hydrogen, a hydroxy group or a short chain alkyl residue; the other contains an alkyl-, alkenyl-, hydroxy-

2067754

- 25 -

alkyl-, hydroxyalkenyl- or hydroxyacyl-chain (or an alkenyl-, alkoxy-, alkenyloxy- or acyloxy-residue) with 8-24 carbon atoms. The R_3 residue, as a rule, corresponds to hydrogen or an alkyl chain with less than 5 carbon atoms. R_4 can be an anionic oxygen or a hydroxy group; an alkyl chain with up to 8 C-atoms can also appear as well as another carbohydrate residue with up to 12 carbon atoms; if R_1 as well as R_2 are hydrogen and/or hydroxy groups, a steroid residue, a sugar derivative, a chain containing an amino group, etc., can also appear. Alkyl residues can also be substituted.

Amongst the most suitable surfactants of this substance class are: n-tetradecyl(=myristoyl)-glycero-phosphatidic acid, n-hexadecyl(=plamityl)-glycero-phosphatidic acid, n-octadecyl(=stearyl)-glycero-phosphatidic acid, n-hexadecylene(=palmitoleil)-glycero-phosphatidic acid, n-octadecylene(=oleil)-glycero-phosphatidic acid, n-tetradecyl-glycero,phosphoglycerol, n-hexadecyl-glycero-phosphoglycerol, n-octadecylene-glycero- phosphoglycerol, n-tetradecyl-glycero-phosphoserine, n-hexadecyl-glycerophosphoserine, -n-octadecyl-glycero-phosphoserine, n-hexadecylene-glycero-phosphoserine and n-octadecylene-glycero-phosphoserine.

The corresponding lyso-sulfolipids, phosphono- or phosphinolipids are also suitable edge active compounds according to this invention.

Counterion in these compounds is most frequently an alkali metal cation (such as lithium, sodium, potassium, cesium) or a water soluble tetraalkylammonium-ion (such as tetramethylammonium, tetrathylammonium, etc.).

All corresponding statements made above for surfactants of basic formula 3 also pertain to the carbohydrate residue R_1 .

This residue in the majority of cases is a straight chain or branched alkyl or alkenoyl chain with 6-24, very frequently 10-20, in particular 12-18, carbon atoms and 1-6, especially frequently 1-3, double bonds in n-3- or n-6- positions.

Very convenient alkyl-residues R_1 or R_2 are, for example, n-dodecyl, n-tetradecyl, n-hexadecyl, n-octadecyl, n-eicosyl or n-docosyl chains. N-nonyl, n-undecyl, n-tridecyl, n-pentadecyl, n-heptadecyl and n-nonadecyl, however, are equally useful.

An alkenyl in position R_1 or R_2 is preferably a 9-cis-dodecenyl (lauroleyl), 9-cis-tetradecenyl (myristoleyl), 9-cis-hexadecenyl (palmitoleoyl), 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl), 11-cis-octadecenyl (vaccenyl), 9-cis-eicosenyl (gadoleinyl), 13-cis-docosenyl, 13-trans-docosenyl or 15-cis-tetracosenyl, etc.

Higher unsaturated alkenyls which also can be used for the present purpose are, amongst others: 9-cis,12-cis-octadecendienyl, 9-trans,12-trans-octadecendienyl, 9-cis,12-cis,15-cis-octadecentrienyl, 6-cis,9-cis,12-cis-octadecentrienyl, 11-cis,14-cis,17-cis-eicosatrienyl, 6-cis,9-cis,12-cis,15-cis-octadecentetraenyl, 5-cis,8-cis,11-cis,14-cis-eicosatetraenyl, 5-cis,8-cis,11-cis,14-cis,17-cis-eicosapentaenyl, 4-cis,7-cis,10-cis,13-cis,16-cis-docosapentaenyl and 4-cis,7-cis,10-cis,13-cis,16-cis,19-cis-docosahexaenyl.

R_1 and R_2 are preferably chosen from the substances of the hydroxyalkyl-class, in which case they correspond, for example, to n-decylhydroxy, n-dodecylhydroxy (hydroxylauryl), n-tetradecylhydroxy (hydroxymyristyl), n-hexadecylhydroxy (hydroxycetyl), n-octadecylhydroxy (hydroxystearyl) and n-

eicosylhydroxy (hydroxyarachinyl) chains.

An alkenylhydroxy in R_1 or R_2 is preferably a 9-cis-dodecenylhydroxy (hydroxylauroleyl), 9-cis-tetradecenylhydroxy (hydroxymyristoleyl), 9-cis-hexadecenylhydroxy (hydroxypalmitoleinyl), 6-cis-octadecenylhydroxy (petroselinylhydroxy), 6-trans-octadecenylhydroxy (hydroxypetroselaidinyl), 9-cis-octadecenylhydroxy (hydroxyoleyl), 9-trans-octadecenylhydroxy (hydroxyelaidinyl) and 9-cis-eicosenyl (hydroxygadoleinyl) chain.

An alkanoylhydroxy in R_1 or R_2 is preferably an n-decanoylhydroxy, n-dodecanoylhydroxy (lauroylhydroxy), n-tetradecanoylhydroxy (myristoylhydroxy), n-hexadecanoylhydroxy, n-hexadecanoylhydroxy (palmitoylhydroxy), n-octadecanoylhydroxy (stearoylhydroxy) and n-eicosoylhydroxy (arachinoylhydroxy) chain.

An alkenoylhydroxy in R_1 or R_2 is preferably a 9-cis-dodecenylhydroxy (lauroleoylhydroxy), 9-cis-tetradecenoylhydroxy (myristoleoylhydroxy), 9-cis-hexadecenoylhydroxy (palmitoleinoylhydroxy), 6-cis-octadecenoylhydroxy (petroselinoylhydroxy), 6-trans-octadecenoylhydroxy (petroselaidinoylhydroxy), 9-cis-octadecenoylhydroxy (oleoylhydroxy), 9-trans-octadecenoylhydroxy (elaidinoylhydroxy) and 9-cis-eicosenoyl (gadoleinoylhydroxy) chain.

Some examples for the short chain alkyl residue, which often appear in the R_4 residue, are methylene-, ethylene-, n-propylene-, iso-propylene-, n-butylene- or iso-butylene- as well as n-pentylene- or n-hexylene-groups. R_4 can also be a carboxy- or a sulfo-group, an acid or alkaline group, such as carboxy- and amino-group; the amino group in such case is always in the alpha-position relative to the carboxy group.

2067754

- 28 -

Another example for the R_4 residue are free or etherified hydroxy groups (two ether-bonded hydroxy groups, in such case, can be connected by one divalent hydrocarbon residue, such as methylene, ethylene, ethylidene, 1,2-propylene or 2,2-propylene). R_4 , furthermore, can be substituted by a halogen atom, such as chlorine or bromine, a low weight alkoxy-carbonyl, such as methoxy- or ethoxycarbonyl, or by a low weight alkansulfonyl-, such as methansulfonyl.

A substituted short chain alkyl residue R_4 with 1-7 C-atoms is preferably carboxy-short-chain alkyl, such as carboxy-methyl, carboxyethyl- or 3-carboxy-n-propyl, omega-amino-n-carboxy- a short-chain alkyl, such as 2-amino-2-carboxyethyl or 3-amino-3-carboxy-n-propyl, hydroxy-short-chain alkyl, such as 2-hydroxyethyl or 2,3-dihydroxypropyl, a short-chain alkoxy-3-methoxy-n-propyl, a short-chain alkylendioxy-short-chain alkyl, such as 2,3-ethylenedioxypropyl or 2,3-(2,2-propylene)-dioxypropyl, or halogen-short-chain alkyl, such as chloro- or bromo-methyl, 2-chloro- or 2-bromo-ethyl, 2- or 3-chloro- or 2-or 3-bromo-n-propyl.

A carbohydrate residue R_4 with 5-12 C-atoms is, for example, a natural monosaccharide residue stemming from a pentose or a hexose in the aldose or ketose form.

* A carbohydrate residue R_4 , moreover, can be a natural disaccharide residue, such as a disaccharide residue formed from two hexoses, in the described sense. A carbohydrate residue R_4 can also be a derivatised mono-, di- or oligosaccharide residue, in which an aldehyde group and/or one or two terminal hydroxy groups are oxidized to a carboxy group, e.g. a D-glucon-, D-glucar- or D-glucoron acid residue; this preferably appears in the form of a cyclic lactone residue. The aldehyde- or keto-groups in a derivatised mono-

or disaccharide residue can also be reduced to a hydroxy group, e.g. in inositol, sorbitol or D-mannitol; also, one or several hydroxy groups can be replaced by a hydrogen atom, e.g. in desoxysugars, such as 2-desoxy-D-ribose, L-rhamnose or L-fucose, or by an amino group, e.g. in aminosugars, such as D-glucosamine or D-galactosamine.

R_4 can also be a steroid residue or a sterine residue. If R_4 is a steroid residue, R_3 is a hydrogen atom, whilst R_1 and R_2 in such case preferably correspond to a hydroxy group.

The counterion in such cases is preferably an ammonium, sodium or potassium ion.

In an anionic surfactant of formula 8, the following values of parameters are preferred: $n = 1$, R_1 is an alkyl, such as n-dodecyl (lauryl), n-tridecyl, n-tetradecyl (myristyl), n-pentadecyl, n-hexadecyl (cetyl), n-heptadecyl or n-octadecyl (stearyl), hydroxyalkyl, such as n-dodecylhydroxy (hydroxy-lauryl), n-tetradecylhydroxy (hydroxymyristyl), n-hexadecylhydroxy (hydroxycetyl), or n-octadecylhydroxy (hydroxystearyl), hydroxyacyl, such as hydroxylauroyl, hydroxymyristoyl, hydroxypalmitoyl or hydroxystearoyl, R_2 is a hydrogen atom or a hydroxy group, R_3 is a hydrogen atom or a short-chain alkyl, such as methyl, R_4 is a short-chain alkyl, e.g. methyl or ethyl, short-chain alkyl substituted by an acid or an alkaline group, such as a carboxy and amino group, e.g. omega-amino-omega-carboxy-short-chain alkyl, such as 2-amino-2-carboxyethyl or 3-amino-3-carboxy-n-propyl, hydroxy-short-chain alkyl, such as 2-hydroxyethyl or 2,3-hydroxypropyl, short-chain alkylenedioxy-short-chain alkyl, e.g. 2,3-ethylenedioxypropyl or 2,3-(2,2-propylene)-dioxypentyl, halogen-short-chain alkyl, such as 2-chloro- or 2-bromo-ethyl group, a carbohydrate residue with 5-12 C-

atoms, e.g. in inositol, or a steroid residue, such as a sterol, e.g. cholesterolin, and G^+ is a sodium-, potassium- or ammonium-ion.

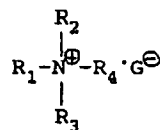
An anionic surfactant of formula 8, in many cases, is a sodium- or potassium salt of lysophosphatidylserine, such as the sodium- or potassium salt of lysophosphatidylserine from bovine brain or the sodium- or potassium salt of a synthetic lysophosphatidylserine, such as sodium- or potassium-1-myristoyl- or -1-palmitoyl-lysophosphatidylserine, or a sodium- or potassium salt of lysophosphatidylglycerols. The hydrogen atom on the phosphate group can be replaced by a second cation, G^+ or calcium-, magnesium-, manganese-ion, etc.

An anionic surfactant of formula 8 preferably contains an alkyl chain, such as n-dodecyl (lauryl), n-tridecyl, n-tetradecyl (myristoyl), n-pentadecyl, n-hexadecyl (cetyl), n-heptadecyl or n-octadecyl (stearyl), a hydroxyalkyl chain, such as n-dodecylhydroxy (hydroxylauryl), n-tetradecylhydroxy (hydroxymyristyl), n-hexadecylhydroxy (hydroxycetyl), or n-octadecylhydroxy (hydroxystearyl), a hydroxyacyl chain, such as hydroxylauroyl, hydroxymyristoyl, hydroxypalmitoyl or hydroxystearyl in position R_1 , a hydrogen atom or a hydroxy group in position R_2 , and a hydrogen atom or a short-chain alkyl, such as methyl group, in position R_3 . G^+ is preferably an ammonium, sodium, potassium or tetramethylammonium ion.

An anionic surfactant of formula 8 can, furthermore, be a sodium- or potassium salt of a natural phosphatidic acid, such as egg-phosphatidic acid, a sodium- or potassium salt of a natural lysophosphatidic acid, such as egg-lysophosphatidic acid, a sodium- or potassium salt of a synthetic lysophosphatidic acid, such as 1-lauroyl-, 1-myristoyl-, 1-palmitoyl- or 1-oleoyl-lysophosphatidic acid, etc.

The most important classes of cationic surfactants encompass: ammonium salts, quarternary ammonium salts, salts of heterocyclic bases, such as alkylpyridium-, imidazole-, or imidazolinium salts, salts of alkylamides and polyamines, salts of acylated diamines and polyamines, salts of acylated alkanolamines, salts of alkanolamine esters and ethers, etc.

A cationic surfactant is, for example, any substance corresponding to the formula:



(9)

in which R_1 is a hydrocarbon residue which can also be substituted. R_2 denotes a short-chain alkyl, phenyl-short-chain-alkyl or hydrogen atom. R_3 and R_4 correspond to a short-chain alkyl residue. R_2 and R_3 , together with the nitrogen atom, represent an aliphatic heterocycle, which can also be substituted on a carbon atom; R_4 is a short-chain alkyl; R_2 , R_3 and R_4 , together with the nitrogen atom, can also form an aromatic heterocycle, which, moreover, can be substituted on one of the carbon atoms. G^- corresponds to an anion.

In a cationic surfactant of basic formula 9, R_1 represents an aliphatic hydrocarbon residue, which can also be substituted, for example, by an aryloxy- short-chain-alkoxy-, a substituted short-chain alkyl, a straight chain or branched chain alkyl with 7-22, and in particular 12-20, carbon atoms, or an alkenyl with 8-20, or in particular 12-20, carbon atoms and 1-4 double bonds.

Particularly preferred for use are straight chain alkyls with an even number of 12-22 carbon atoms, such as n-dodecyl, n-tetradecyl, n-hexadecyl, n-octadecyl, n-eicosyl or n-docosyl.

An alkenyl with 8-24, in particular 12-22, carbon atoms and 0-5, in particular 1-3, double bonds is e.g. 1-octenyl, 1-nonenyl, 1-decenyl, 1-undecenyl, 1-dodecenyl, 9-cis-dodecenyl (lauroleyl), 1-tridecenyl, 1-tetradecenyl, 9-cis-tetradecenyl (myristoleyl), 1-pentadecenyl, 1-hexadecenyl, 9-cis-hexadecenyl (palmitoleinyl), 1-heptadecenyl, 1-octadecenyl, 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl), 9-cis-12-cis-octadecadienyl (linoleyl), 9-cis-11-trans-13-trans-octadecatrienyl (alpha-elaostearinyl), 9-trans-11-trans-13-trans-octadecatrienyl (beta-elaostearinyl), 9-cis-12-15-cis-octadecatrienyl (linolenyl), 9-, 11-, 13-, 15-octadecatetraenyl (parinaryl), 1-nonadecenyl, 1-eicosenyl, 9-cis-eicosenyl (gadoleinyl), 5-, 11-, 14-eicosatrienyl or 5-, 8-, 11-, 14-eicosatetraenyl (arachidonyl).

Preferred alkenyls contain 12-20 carbon atoms and one double bond, e.g. 9-cis-dodecenyl (lauroleyl), 9-cis-tetradecenyl (myristoleyl), 9-cis-hexadecenyl (palmitoleinyl), 6-cis-octadecenyl (petroselinyl), 6-trans-octadecenyl (petroselaidinyl), 9-cis-octadecenyl (oleyl), 9-trans-octadecenyl (elaidinyl) or 9-cis-eicosenyl (gadoleinyl).

Methyl or ethyl are two examples of short-chain alkyl residues R_2 , R_3 or R_4 which appear in substances of formula 9.

Two examples of phenyl-short-chain-alkyl groups in R_2 are benzyl or 2-phenylethyl.

An aliphatic heterocycle, which can form from R_2 and R_3 together with the nitrogen atom is, for example, a monocyclic, five- or six-member aza-, oxaaza- or thiazaacyclic residue, as in piperidino, morpholino or thiamorpholinio groups.

Substituents of this heterocycle are the substituents R_1 and R_4 on the nitrogen as well as, in some cases, on the carbon atom; they are, most frequently, of the short-chain alkyl, such as methyl, ethyl, n-propyl or n-butyl type.

•

A heterocycle, which is formed from R_2 and R_3 together with nitrogen and is substituted on a carbon atom through a short-chain alkyl, is e.g. of the 2-, 3- or 4-methylpiperidinio, 2-, 3- or 4-ethylpiperidinio or 2- or 3-methylmorpholinio type.

An aromatic heterocycle, formed from R_2 , R_3 and R_4 together with the nitrogen atom, is, for example, a monocyclic five- or six-member aza-, diaza-, oxaaza- or thiazaacyclic residue, such as pyridinio, imidazolinio, oxazolinio or thiazolinio or, for example, a benzocondensed monoazabicyclic residue, such as chinolinio or iso-chinolinio group.

Substituents of such heterocycles are the residue R_1 on the nitrogen atom as well as a short-chain alkyl, such as methyl or ethyl, hydroxy-short-chain alkyl, such as hydroxymethyl or 2-hydroxyethyl, oxo-, hydroxy- or halogen, such as chloro- or bromo-compounds, which can also be substituted on a carbon atom.

A heterocycle, formed from R_2 , R_3 and R_4 and substituted on a carbon atom through the mentioned residues is, for example, a 2- or 4-short-chain-alkylpyridinio, e.g. 2- or 4-methyl or 2- or 4-ethylpyridinio, di-short-chain-alkylpyridinio, e.g. 2,6-

2067754

- 34 -

dimethyl-, 2-methyl-3-ethyl-, 2-methyl-4-ethyl-, 2-methyl-5-ethyl-, or 2-methyl-6-ethylpyridinio, 2-, 3- or 4-halogenpyridinio, e.g. 2-, 3- or 4-chloropyridinio or 2-, 3- or 4-bromo-pyridinio, 2-short-chain alkylimidazolinio, -oxazolinio or -thiazolinio, such as 2-methyl- or 2-ethylimidazolinio, -oxazolinio or -thiazolinio or 2-short-chain alkyl-8-halogenchinolinio, such as 2-methyl-8-chlorochinolinio group.

A cationic surfactant of formula 9 is preferably an N-benzyl-N,N-dimethyl-N-2-(2-(4-(1,1,3,3-tetramethylbutyl)-phenhydroxy)-ethhydroxy)-ethylammoniochloride, N-benzyl-N,N-dimethyl-N-2-(2-(3(methyl-4-(1,1,3,3-tetramethylbutyl)-phenhydroxy)-ethhydroxy)-ethylammoniochloride (methylbenzethoniumchloride), n-dodecyltrimethylammoniochloride or -bromide, trimethyl-n-tetradecylammoniochloride or -bromide, n-hexadecyltrimethylammoniochloride or -bromide (cetyltrimethyl-ammoniumchloride or -bromide), trimethyl-n-octadecylammoniochloride or -bromide, ethyl-n-dodecyl-dimethylammoniochloride or -bromide, ethyldimethyl-n-tetradecylammoniochloride or -bromide, ethyl-n-hexadecyldimethylammoniochloride or -bromide, ethyldimethyl-n-octadecylammoniochloride or -bromide, n-alkyl-benzyl-dimethyl-ammoniochloride or -bromid (benzalkoniumchloride or -bromide), such as benzyl-n-dodecyldimethylammoniochloride or bromide, benzyldimethyl-n-tetradecylammoniochloride or -bromide, benzyl-n-hexadecyldimethyl-ammoniochloride or -bromide or benzyldimethyl-n-octadecylammonio-chloride or -bromide, N-(n-decyl)-pyridiniochloride or -bromide, N-(n-dodecyl)-pyridiniochloride or -bromide, N-(n-tetradecyl)-pyridiniochloride or -bromide, N-(n-hexadecyl)-pyridiniochloride or -bromide (cetylpyridiniumchloride) or N-(n-octadecyl)-pyridinio-chloride or -bromide. Mixtures of these or other edge active substances are also suitable.

The following surfactants are especially useful for biological

2067754

- 35 -

purposes: N,N-bis(3-D-glucon-amidopropyl)cholamide (BigCHAP), Bis(2-ethylhexyl)sodium-sulfosuccinate, cetyl-trimethylammonium-bromide, 3-((cholamidopropyl)-dimethylammonio)-2-hydroxy-1-propane sulfonate (CHAPSO), 3-((cholamidopropyl)-dimethylammonio)-1-propane sulfonate (CHAPS), cholate-sodium salt, decaoxyethylene-dodecyl-ether (Genapol C-100), decaethylene-isotridecyl-ether (Genapol X-100), decanoyl-N-methyl-glucamide (MEGA-10), decyl-glucoside, decyl-maltoside, 3-(decyldimethylammonio)-propane-sulfonate (Zwittergent 3-10), deoxy-bigCHAP, deoxycholate, sodium salt, digitonin, 3-(dodecyldimethylammonio)-propane-sulfonate (Zwittergent 3-12), dodecyl-dimethyl-amine-oxide (EMPIGEN), dodecyl-maltoside, dodecylsulfate, glyco-cholate, sodium salt, glyco-deoxycholate, sodium salt, heptaethylene-glycol-octyl-phenyl-ether (triton X-114), heptyl-glucoside, heptyl-thiogluconoside, 3-(hexadecyldimethylammonio)-propane-sulfonate (Zwittergent 3-14), hexyl-glucoside, dodecyl-dimethyl-amine-oxide (Genaminox KC), N-dodecyl-N,N-dimethylglycine (Empigen BB), N-decyl-sulfobetaine (Zwittergent 3-10), N-dodecyl-sulfobetaine (Zwittergent 3-12), N-hexadecyl-sulfobetaine (Zwittergent 3-16), N-tetradecyl-sulfobetaine (Zwittergent 3-14), N-octyl-sulfobetaine (Zwittergent 3-08), nonaethylene-glycol-mono-dodecyl-ether (THESIT), nonaethylene-glycol-octyl-phenol-ether (triton X-100), nonaethylene-glycol-octyl-phenyl-ether (NP-40, Nonidet P-40), nonaethylene-dodecyl-ether, nonanoyl-N-methyl-glucamide (MEGA-9), nonaoxyethylene-dodecyl-ether (Lubrol PX, Thesit), nonyl-glucoside, octaethylene-glycol-isotridecyl-ether (Genapol X-080), octaethylene-dodecyl-ether, octanoyl-N-methyl-glucamide (MEGA-8), 3-(octyldimethylammonio)-propane-sulfonate (Zwittergent 3-08), octyl-glucoside, octyl-thiogluconoside, entadecaethylene-isotridecyl-ether (Genapol X-150), polyethylene-polypropylene-glycol (Pluronic F-127), polyoxyethylene-sorbitane-monolaurate (Tween 20), polyoxyethylene-sorbitane-monooleate (Tween 80), taurodeoxycholate-sodium salt, taurocholate-sodium salt, 3-(tetradecyl-

2067754

- 36 -

dimethylammonio)-propane-sulfonate (Zwittergent 3-14), etc.

Particularly suitable for pharmacological purposes are:

cetyl-trimethyl-ammonium-salts (such as hexadecyltrimethylammoniumbromide, trimethylhexadecylamine-bromo-salt), cetylsulfate salts (such as Na-salt, Lanette E), cholate salts (such as Na- and ammonium-form) decaoxyethylene-dodecyl-ether (Genapol C-100), deoxycholate salts, dodecyl-dimethyl-amine-oxide (Genaminox KC, EMPIGEN), N-dodecyl-N,N-dimethylglycine (Empigen BB), 3-(hexadecyldimethylammonio)-propane-sulfonate (Zwittergent 3-14), fatty acid salts and fatty alcohols, glyco-deoxycholate salts, laurylsulfate salts (sodium dodecylsulfate, Duponol C, SDS, Texapon K12), N-hexadecyl-sulfobetaine (Zwittergent 3-16), nonaethylene-glycol-octyl-phenyl-ether (NP-40, Nonidet P-40), nonaethylene-dodecyl-ether, octaethylene-glycol-isotridecyl-ether (Genapol X-080), octaethylene-dodecyl-ether, polyethylene glycol-20-sorbitane-monolaurate (Tween 20), polyethylene glycol-20-sorbitane-monostearate (Tween 60), polyethylene glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-cetylstearyl ether (Cetomacrogol, Cremophor O, Eumulgin, C 1000) polyhydroxyethylene-4-lauryl ether (Brij 30), polyhydroxyethylene-23-lauryl ether (Brij 35), polyhydroxyethylene-8-stearate (Myrj 45, Cremophor AP), polyhydroxyethylene-40-stearate (Myrj 52), polyhydroxyethylene-100-stearate (Myrj 59), polyethoxylated castor oil 40 (Cremophor EL), polyethoxylated hydrogenated castor oil (Cremophor RH 40, Cremophor RH 60) polyethoxylated plant oils (Lebrafils), sorbitane-monolaurate (Arlacel 20, Span 20), taurodeoxycholate salts, taurocholate salts, polyethylene glycol-20-sorbitane-palmitate (Tween 40), Myrj 49 and polyethylene glycol derivatives of ricinols, etc.

AGENTS:

Transfersomes as described in this invention are suitable for the application of many different agents and, in particular, for therapeutic purposes, for example. The preparations according to this invention can contain the following:

- at least one adrenocorticostatic agent, in particular metyrapon;
- at least one carrier substance, additive or agent, belonging to the class of beta-adrenolytics (beta blocking agents), very frequently acetobol, alprenolol, bisoprololfumarate, bupranolol, carazolol, celiprolol, mepindolsulfate, metipranolol, metoprolotartat, nadolol, oxyprenolol, pindolol, sotalol, tertatolol, timolohydrogen maleate and toliprolol, especially preferred, atenolol or propranolol;
- at least one carrier substance, additive or agent, belonging to the androgenes or antiandrogenes, in particular drostanolonpropionate, mesterolone, testosteronundecanoate, testolacton, yohimbine, or chloroamidinonacetate, cyproteronacetate, ethinylestradiol or flutamide;
- at least one carrier substance, additive or agent with an antiparasitic action, frequently phanquinone, benzyobenzoate, bphenium-hydroxy-naphthoate, crotamiton, diethylcarbamazine, levamisol, lindane, malathione, mesulfene (2,7-dimethylantren), metronidazol or tetramisol;
- at least one anabolic agent, in particular clostebolacetate, cyanocobolamine, folic acid, mestanolone, metandienone, metenolone, nandrolone, nandrolondecanoate, nandrolone-hexyloxyphenylpropionate,

2067754

- 38 -

nandrolon-phenyl-propionate, norethandrolone,
oxaboloncipionate, piridoxine or stanozolole;

- at least one agent which can induce systemic anesthesia or analgesia, e.g. chlorobutanol, ketamine, oxetacaine, propanidide and thiamylal, aminophenol-derivatives, aminophenazol-derivatives, antranilic acid- and arylpropione acid derivatives, azapropazone, bumadizone, chloroquin- and codeine-derivatives, diclophenac, fentanil, ibuprofen, indometacine, ketoprofen, methadone-substances, morazone, morphine and its derivatives, nifenazone, niflumin acid, pentazozine, pethidine, phenazopyridine, phenylbutazone-derivatives (such as 3,5 pyrazolidine dion), pherazone, piroxicam, propoxyphene, propyphenazon, pyrazol- and phenazone-derivatives (aminophenazone, metamizole, monophenylbutazone, oxyphenebutazone, phenylbutazone or phenazone-salyzilate), salicylic acid-derivatives, sulfasalazine, tilidine; acetylsalicylic acid, ethylmorphine, alclofenac, alphaprodine, aminophenazone, anileridine, azapropazone, benfotiamine, benorilate, benzydamine, cetobemidone, chlorophenesincarbamate, chlorothenoxazine, codeine, dextromoramide, dextro-propoxyphene, ethoheptazine, fentanyl, fenylramidol, fursultiamine, flupirtinmaleate, glafenine, hydromorphone, lactylphenetidine, levorphanol, mefenamic acid, meptazonol, methadone, mofebutazone, nalbufine, Na-salt of noramidopyrinium-methanesulfonate, nefopam, normethadone, oxycodone, paracetamol, pentazocine, pethidine, phenacetine, phenazocine, phenoperidine, pholcodine, piperylone, piritramide, procaine, propyphenazone, salicylamide, thebacone, tiemonium-odide, tramadone;
- at least one substance from the class of analeptics, such

as aminophenazole, bemegride, caffeine, doxapram, ephedrine, prolintane, or nialamide and tranylcypromine; but also vitamins, plant extracts from semen colae, camphor, menthol;

- at least one substance from the class of antiallergics: e.g. agents from the globuline family, corticoids or antihistaminics (such as beclomethasone-, betametasone-cortisone-, dexametasone-derivatives, etc.) as well as bamipinacetate, buclizine, clemastine, clemizole, cromoglicinic acid, cyproheptadine, diflucorolonvalerate, dimetotiazine, diphenhydramine, diphenylpyraline, ephedrine, fluocinolane, histapyrrodine, isothipendyle, methadilazine, oxomemazine, paramethasone, prednilidene, theophylline, tolpropamine tritoqualine, etc. are used; amongst the preferred agents in this class are the substances characterized by their capacity to interfere (stimulate or suppress) the production of immunologically active substances, such as interleukines, interferones, leucotrienes, prostaglandines, etc. Amongst others, certain lipids and lipoids, such as phosphatidylcholines and diacylglycerols, or fatty acids and their esters, with chains containing several, preferably 3-6, most very frequently 3 or 4, double bonds, preferably of the n-3 type, are used for this purpose; the latter may also be hydroxygenated, branched or (partially) derivatized into ring structures.
- at least one substance with antiarrhythmic action, such as most of the cardiacs and beta-blockers, ajmaline, bupranolol, chinidine, digoxine derivatives, diltiazem, disopyramidedihydrogensulfate, erythromycine, disopyramide, gallopamil, ipratropiumbromide, lanatoside, lidocaine, lorcaïnide, orciprenalinesulfate, procaine amide, propafenone, sparteineosulfate, verapamil,

toliprolol.

- an antiarteriosclerotic, such as clofibrate.
- at least one substance belonging to the antiasthmatics and/or bronchospasmolytics, such as amiodarone, carbuterol, fenoterol, orciprenalin, sotalol, or theophylline-derivatives, as well as corticoids (such as beclomethasone, dexamethasone, hydrocortisone, prednisolone), frequently in combination with purines;
- at least one substance from the class of antibiotics, such as actinomycine, alamethicine, alexidine, 6-aminopenicillanic acid, moxycilline, amphotericine, ampicilline, anisomycine, antiamoebine, antimycine, aphidicoline, azidamfenicol, azidocilline, bacitracine, beclomethasone, benzathine, benzylpenicilline, bleomycine, bleomycine sulfate, calcium ionophor A23187, capreomycine, carbenicilline, cefacetril, cefaclor, cefamandole nafate, cefazoline, cefalexine, cefaloglycine, cefaloridine, cefalotine, cefapirine, cefazoline, cefoperazone, ceftriaxone, cefuroxim, cephalaxine, cephaloglycine, cephalothine, cephapirine, cerulenine, chloroamphenicol, chlorotetracycline, chloroamphenicol diacetate, ciclaciline, clindamycine, chloromadinone acetate, chloropheniramine, chromomycine A3, cinnarizine, ciprofloxacin, clotrimazole, cloxacilline, colistine methanesulfonate, cycloserine, deacetylanisomycine, demeclocycline, 4,4'-diaminodiphenyl sulfone, diaveridine, dicloxacilline, dihydrostreptomycine, dipyridamol, doxorubicine, doxycycline, epicilline, erythromycine, erythromycine-stolate, erythromycinethylsuccinate, erythromycine stearate, ethambutol, flucloxacilline, fluocinolone acetonide, 5-fluorocytosine, filipine, formycine,

fumaramidomycine, furaltadone, fusidic acid, geneticine, gentamycine, gentamycine sulfate, gliotoxine, gfamicidine, griseofulvine, helvolic acid, hemolysine, hetacillin, kasugamycine, kanamycine (A), lasalocide, lincomycine, magnesidine, melphalane, metacycline, meticilline, mevinoline, micamycine, mithramycine, mithramycine A, mithramycine complex, mitomycine, minocycline, mycophenolic acid, myxothiazol, natamycine, nafcilline, neomycine, neomycine sulfate, 5-nitro-2-furaldehydesemicarbazone, novobiocine, nystatine, oleandomycine, oleandomycine phosphate, oxacihine, oxytetracycline, paromomycine, penicilline, pecilocine, pheneticilline, phenoxymethylpenicilline, phenyl amino-salicylate, phleomycine, pivampicilline, polymyxine B, propicilline, puromycine, puromycine aminonucleoside, puromycine aminonucleoside 5'-monophosphate, pyridinol carbamate, rolitetracycline, rifampicine, rifamycine B, rifamycine SV, spectinomycine, spiramycine, streptomycine, streptomycine sulfate, sulfabenzamide, sulfadimethoxine, sulfamethizol, sulfamethoxazol, tetracycline, thiamphenicol, tobramycine, troleandomycine, tunicamycine, tunicamycine A1-homologs, tunicamycine A2-homolog, valinomycine, vancomycine, vineomycine A1, virginiamycine M1, viomycine, xylostasine;

at least one substance with an antidepressive or antipsychotic action, such as diverse monoaminoxidase-suppressors, tri- and tetracyclic antidepressives, etc. Very frequently used agents of this class are alprazolame, amitriptyline, chlorpromazine, clomipramine, desipramine, dibenzepine, dimetacrine, dosulepine, doxepine, fluvoxaminhydrogenmaleate, imipramine, isocarboxazide, lofepramine, maprotiline, melitracene, mianserine, nialamide, noxiptiline,

nomifensine, nortriptyline, opipramol, oxypertine, oxytriptane, phenelzine, protriptyline, sulpiride, tranylcypromine, trosadone, tryptophane, vitoxazine, etc.

- at least one antidiabetic agent, such as acetohexamide, buformine, carbutamide, chloropropamide, glibenclamide, glibornuride, glymidine, metformine, phenformine, tolazamide, tolbutamide;
- at least one substance acting as an antidote, for example, against the heavy metal poisoning, poisoning with insecticides, against drugs, blood poisons, etc. A few examples are different chelators, amiphenazol obidoxim-chloride, D-penicillamine, tiopromine, etc.;
- at least one substance from the class of antiemetics: some of such suitable agents are alizapride, benzquinamide, betahistidine-derivatives, cyclizine, difenidol, dimenhydrinate, haloperidol, meclozine, metoclopramide, metopimazine, oxypendyl, perphenazine, pipamazine, piprinhydrate, prochloroperazine, promazine, scopolamine, sulpiride, thiethylperazine, thioproperazine, triflupromazine, trimethobenzamide, etc., which are frequently used in combination with vitamins and/or antiallergics;
- at least one substance with an antiepileptic action, such as barbexaclone, barbiturate, beclamide, carbamazepine, chloroalhydrate, clonazepam, diazepam, ethosuximide, ethylphenacemide, lorazepam, mephenytoine, mesuximide, oxazolidine, phenaglycodol, phensuximide, phenytoine, primidone, succinimide-derivatives, sultiam, trimethadione, yalproinic acid, etc.; additives are commonly chosen from the classes of hypnotics and sedatives; an especially commonly used agent of this kind is

2067754

- 43 -

carbamazepine.

- at least one substance with antifibrinolytic activity, such as aminocaproic acid or tranexamic acid.
- at least one anticonvulsive agent, such as beclamide, carbamazepine, clomethiazole, clonazepam, methylphenobarbital, phenobarbital or sultiam;
- at least one substance which modifies choline concentration, by having an anticholinergic activity, for example. The following substances can be used, amongst others, as cholinergics: aubenoniumchloride, carbachol, cerulezide, dexpanthenol and stigmine-derivatives (such as distigminebromide, neostigminemethylsulfate, pyridostigmine-bromide); frequently used as anticholinergics are especially atropine, atropinmethonitrate, benactyzine, benzilonium-bromide, bevonium-methylsulfate, chlorobenzoxamine, ciclonium-bromide, clidinium-bromide, dicycloverine, diphemanil-methylsulfate, fempiverinium-bromide, glycopyrroniumbromide, isopropamide-iodide, mepenzolate-bromide, octatropine-methylbromide, oxyphencyclimine, oxyphenonium-bromide, pentapiperide, pipenzolate-bromide, piperidolate, pridinol, propanidide, tridihexethyl-iodide and trospiumchloride; cholinesterase inhibitors, such as ambenonium-chloride, demecarium-bromide, echothiopate-iodide, etc., are also useful for this purpose;
- at least one substance which can change, in the majority of cases diminish, the effect or concentration of histamine (antihistaminics). Preferred are hypoallergic carriers or hypoallergic edge active substances with n-3 (omega-3), less frequently with n-6 (omega-6), and mainly several, often 3-6 double bonds; such substances are

occasionally employed with hydroxy, more rarely methyl-, or oxo-side groups, or in an epoxy configuration; further suitable agents of this class are, among other substances, aethylenediamine, alimemazine, antazoline, bamipine, bromo-azine, bromo-pheniramine, buclizine, carbinoxamine, chlorocyclizine, chloropyramine, chlorophenamine, chlorophenoxamine, cimetidine, cinnarizine, clemastine, clemizol, colamine (such as diphenhydramine), cyclizine, dexbrompheniramine, dexchloropheniramine, difenidol, dimetindene, dimetotiazine, diphenhydramine, diphenylpyraline, dixyrazine, doxylamine, histapyrrodine, isothipendyl, mebhydroline, meclozine, medrylamine, mepyramine, methdilazine, pheniramine, piperacetazine, piprinhydrinate, pyrilamine (mepyramine), promethazine, propylamine, pyrrobutanine, thenalidine, tolpropamine, tripelennamine, triprolidine, etc.;

- at least one substance belonging to the class of antihypertensives, such as many alpha-receptor agonists, aldosterone-antagonists, angiotensine-converting-enzyme-blockers, antisymphaticotonics, beta-blockers, calcium-antagonists, diuretics, vasodilators, etc.; suitable agents for this purpose are for example alpenolol, atenolol, bendroflumethiazide, betanidine, butizide, chlorotalidone, clonidine, cycletanine, cyclopenthiiazide, debrisoquine, diazoxide, dihydralazine, dihydroergotaminmethanesulfonate, doxazinmesilate, guanethidine, guanoclor, guanoxane, hexamethonium-chloride, hydralazine, labetalol, mecanylanine, methyl dopa, pargyline, phenoxybenzamine, prazosine, quinethazone, spironolactone, bescinnamine, reserpine, trichloromethiazide or vincamine;
- at least one substance which is an inhibitor of biological activity, such as actinomycine C1, alpha-

amanitine, ampicilline, aphidicoline, aprotinine, calmidazolium (R24571), calpaine-inhibitor I, calpaine-inhibitor II, castanospermine, chloroamphenicol, colcemide, cordycepine, cystatine, 2,3-dehydro-2-desoxyn-acetyl-neuraminic acid, 1-desoxymannojirimycine-hydrochloride, 1-desoxynojirimycine, diacylglycerol-kinase-inhibitor, P1, P5-di(adenosine-5')-penta-phosphate, ebelactone A, ebelactone B, erythromycine, ethidiumbromide, N-hydroxyurea, hygromycine B, kanamycine sulfate, alpha2-macroglobuline, N-methyl-1-desoxynojirimycine, mitomycine C, myxothiazol, novobiocine, phalloidine, phenylmethanesulfonylfluoride, puromycine-dihydrochloride, rifampicine, staurosporine, streptomycine sulfate, streptozotocine, G-strophanthine, swainsonine, tetracycline-hydrochloride, trifluoperazine-dihydrochloride, tunicamycine, etc.; useful proteinase inhibitors are, for example, (4-amidinophenyl)-methanesulfonylfluoride (APMSF), antipaine-dihydrochloride, antithrombine III, alpha-1-antitrypsine, aprotinine, bestatine, calpaine-inhibitor I, calpaine-inhibitor II, L-1-chloro-3-(4-tosylamido)-7-amino-2-heptanone-hydrochloride (TLCK), L-1-chloro-3-(4-tosylamido)-4-phenyl-2-butanone (TPCK), chymostatine, cystatine, 3,4-dichlorisocoumarin, E 64, selastatinal, hirudin, kallikrein-inhibitor (aprotinine) L-leucinthiol, leupeptine, pepstatine, phenylmethanesulfonylfluoride (PMSF), phosphoramidone, TLCK (tosyl-lysine-chloromethylketone), TPCK (tosyl-phenylalanine-chloromethylketone), trypsin-inhibitors, etc.;

- at least one substance acting as an antihypotonic agent; quite frequently the corresponding drugs are from the classes of analeptics, cardiacs or corticoids. Suitable agents for this purpose are, for example, angiotensinamide, cardaminol, dobutamine, dopamine, etifelmine,

etilefrine, gepefrine, heptaminol, midodrine, oxedrine, etc., especially norfenefrine;

- at least one substance from the group of anticoagulants. Among other substances, some coumarin-derivatives are suitable for this purpose, as well as heparine and heparinoids, hirudine and related substances, dermatan-sulfate etc.; most frequently used agents of this class are acenocoumarin, anisindione, diphenadione, ethyl-biscoumacetate, heparine, hirudine, phenprocoumon, as well as warfarine;
- at least one substance from the class of antimycotics; well-suited examples of such agents include: amphotericine, bifanozol, buclosamide, chinoline-sulfate, chloromidazol, chlorophenesine, chloroquinaldol, clodantoine, cloxiquine, cyclopiroloxadine, dequaliniumchloride, dimazol, fenticlor, flucytosine, griseofulvine, ketoconazol, miconazol, natamycine, sulbentine, tioconazol, tolnaftate, etc.; particularly frequently, amphotericine, clotrimazol or nystatine are likely to be used for this purpose;
- at least one substance from the class of antimusculars, such as pyridostigmine-bromide;
- at least one substance which is active against morbus parkinson, such as amantadine, benserazide, benztropine, biperidene, cycrimine, levodopa, metixene, orphenadrine, phenglutarimide, pridinol, procyclidine, profenamine or trihexyphenidyl;
- at least one substance with an antiphlogistic activity, such as aescine, acetylsalicylic acid, alclofenac, aminophenazone, azapropazone, benzydamine, bumadizone,

chlorothenoxazine, diclofenac, flufenaminic acid, glafenine, ibuprofene, indometacine, kebuzone, mefenam acid, metiazic acid, mesalazine, mofebutazone, naproxene, niflumine acid, salts, such as Na-salt, noramido-pyriminium-methane-sulfonate, orgoteine, oxyphenbutazone, phenylbutazone, propyphenazone, pyridoxine, tolmetine, etc.; very suitable is, for example, ibuprofen; some of the agents commonly used as antiphlogistics also exhibit an antihistaminic or analgetic activity and belong to the classes of corticoids, vasoactiva, opthalmics or otologics;

- at least one substance which is an antipyretic, such as acetylsalicylic acid, alclofenac, aminophenazone, benzydamine, bumadizone, chinine, chlorinethenoxazine, lactylphenetidine, mepro, paracetamol, phenacetine, propyphenazone or salicylamide;
- at least one substance with an antirheumatic activity, such as acetylsalicylic acid, benorilate, chloroquine, diclofenac, fenoprofene, flufenaminic acid, ibuprofene, kebuzone, lactylphenetidine, mefenamic acid, mofebutazone, naproxene, sodiumaurothiomalate, nifenazone, nifluminic acid, D-penicillamine and salicylamide. Edge active substances, carriers and/or agents, with a hypoallergic action, for example from the groups of analgetics, corticoids and glucocorticoids, enzymes or vitamins, etc., are preferred for this purpose, as well as antiphlogistics, such as quinine, nicotinic acid-, nonylic acid-, or salicylic acid-derivatives, meprobamate, etc.;
- at least one antiseptic such as acriflaviniumchloride, cetalkonium-chloride, cetylpyridinium-chloride, chlorohexidine, chloroquinaldol, dequaliniumchloride,

domiphen-bromide, ethacridine, hexetidine, merbromine, nitrofurazone, oxyquinol, phanquinone, phenazopyridine or phenylmercuriborate, as well as fatty acids with an uneven number of carbon atoms;

- at least one respiratory analeptic or respiration stimulant, such as amiphenazol, ascorbic acid, caffeine, cropropamide, crotethamide, etamivane, ephedrine, fominobene, nicethamide; or aminophenazol and doxaprame, for example;
- at least one broncholytic, such as bamifylline, beclometasone, dexometasone (e.g. in dexometasone-21-isonicotinate), diprophylline, ephinedrine (e.g. in ephinedrinehydrogentartrate), fenoterol, hexoprenaline, ipratropium-bromide, iso-etarine, isoprenaline, orciprenaline, protocylol, proxyphylline, reproterol, salbutamol, terbutaline, tetroquinol, theophylline, etc.; and biological extracts, for example from anis, eucalyptus, thyme, etc.;
- one cardiotonic, especially aminophylline, benfurodilhemisuccinate, etofylline, heptaminol, protheobromine or proxyphylline;
- at least one substance from the class of chemotherapeutic agents, for example, acediasulfone, acriflavinium-chloride, ambazone, dapsone, dibrompropamidine, furazolidone, hydroxymethylnitrofurantoin, idoxuridine, mafenide and sulfateolamide, mepacrine, metronidazol, nalidixinic acid, nifuratel, nifuroxazide, nifuarazine, nifurtimox, ninorazol, nitrofurantoin, oxolinic acid, pentamidine, phenazopyridine, phthalylsulfatehiazole, pyrimethamine, salazosulfapyridine, sulfacarbamide, sulfacetamide, sulfachloropyridazine, sulfadiazine,

sulfadicramide, sulfadimethoxine, sulfaethidol, sulfafurazol, sulfaguanidine, sulfaguanol, sulfamethizol, sulfamethoxazol and cotrimoxazol, sulfamethoxydiazine, sulfamethoxypyridazine, sulfamoxol, sulfanilamide, sulfaperine, sulfaphenazol, sulfatehiazol, sulfisomidine, tinidazol, trimethoprim, etc.;

- at least one substance from the class of coronary dilators, such as bamifylline, benziodarone, carbochromes, dilazep, dipyridamol, etafenone, fendiline, hexobendine, imolamine, lidoflazine, nifedipine, oxyfedrine, pentaerythrityltetranitrate, perhexiline, prenylamine, propatylnitrate, racefemine, trolnitrate, verapamil, visnadine, etc.;
- at least one cytostatic, for example, from the group of alkylating agents, antibiotics, platinum compounds, hormones and their inhibitors, interferones, etc.; very frequently used substances of this kind are:
aclarubicine, azathioprine, bleomycine, busulfane, calciumfolinate, carboplatinum, carmustine, chloroambucil, cis-platinum, cyclophosphamide, cyt-arabine, daunorubicine, epirubicine, fluorouracil, fosfestrol, hydroxycarbamide, ifosfamide, lomustine, melphalane, mercaptopurine, methotrexate, mitomycine C, mitopodozide, mitramicyne, nimustine, pipobromane, prednimustine, procarbazine, testolactone, theosulfane, thiotepa, tioguanine, triaziquone, trofosfamide, vincristine, vindesine, vinblastine, zorubicine, etc.;
- an intestinal antiseptic, such as broxyquinoline, clioquinol, diodohydroxyquinoline, halquinol, etc.;
- at least one diuretic, such as acetazolamide, aminophylline, bendroflumethiazide, bumetanide, butizide,

2067754

- 50 -

chloroazanile, chloromerodrine, chlorothiazide, chloro-
talidone, clopamide, clorexolone, cyclopenthiazide,
cyclothiazide, etacrynic acid, furosemide, hydrochloro-
thiazide, hydroflumethiazide, mefruside, methazolamide,
paraflutizide, polythiazide, quinethazone, spirono-
lactone, triamterene, trichloromethiazide, xipamide,
etc.;

- at least one ganglion blocker, such as gallamintri-
ethiodide, hexamethonium-chloride, mecamlamine, etc.;
- at least one substance for the therapy of arthritis,
preferably analgetics or for example allopurinol,
benzbromarone, colchicine, benziodarone, probenecide,
sulfinpyrazone, tenoxicam, etc.; in very many cases
allopurinol;
- at least one glucocorticoid, such as beclomethason,
betamethason, clocortolone, cloprednol, cortison, dexamethason (e.g. as a dexamethasonephosphate), fludrocortison, fludroxycortide, flumetason, fluocinolon-acetonide, fluocinonide, fluocortolon (e.g. as a fluocortoloncapronate or fluocortolontrimethylacetate), fluorometholon, fluprednidenacetate, hydrocortison (also as a hydrocortison-21-acetate, hydrocortison-21-phosphate, etc.), paramethason, prednisolon (e.g. in the form of methylprednisolon, prednisolon-21-phosphate, prednisolon-21-sulfobenzoate, etc.), prednison, prednyli-den, pregnenolon, triamcinolon, triamcinolonacetonide, etc.;
- at least one agent with a putative anti-flew action, such as moroxydine;
- at least one haemostatic, such as adrenalon, ascorbic

acid, butanol, carbazochrome, etamsylate, protamine, samatostatine etc.; thyroidal hormones and vitamins can be employed for this purpose as well;

- at least one hypnotic, from the class of barbiturates, benzodiazepines, bromo-compounds, ureids, etc., for example; quite commonly applied for this purpose are, e.g. acecarbromal, alimemazintartrate allobarbital, amobarbital, aprobarbital, barbital, bromo-isoval, brotizolam, carbromal, chloroalhydrate, chloroalodol, chlorobutanol, clomethiazol, cyclobarbital, diazepam, diphenhydramine, doxylamine, estazolam, ethchlorvynol, ethinamate, etomidate, flurazepam, glutethimide, heptabarb, hexobarbital, lormetazepam, malperol, meclozine, medozine, methaqualon, methypylon, midazolam, nitrazepam, oxazepam, pentobarbital, phenobarbital, promethazine, propallylonal, pyrithyldion, secbuta-barbital, secobarbital, scopolamine, temazepam, triazolam, vinylbital, etc.; various extracts from balm-mint, valerian, and passiflora are also used;
- at least one immunoglobuline, from the IgA, IgE, IgD, IgG, IgM classes or an immunoglobuline fragment, such as a Fab- or Fab2-fragment, or the corresponding variable or hypervariable region, if required in combination with other agents and/or chemically, biochemically or genetically manipulated;

An immunoglobuline can be of the IgA, IgD and IgE, IgG (e.g. Ig G1, Ig G2, Ig G3, Ig G4) or IgM type. In the context of this application, any chemical or biochemical derivative of any immunoglobuline (Ig) is considered useful, for example, an Ig G-gamma chain, an Ig G-F(ab')₂ fragment, an Ig G-F(ab) fragment, an Ig G-Fc fragment, an Ig-kappa chain, a light chain of Ig-s (e.g. a kappa and

lambda chain), but also even smaller immunoglobuline fragments, such as the variable or hypervariable regions, or artificial modifications of any of these substances.

- at least one substance with an immunostimulating activity, with an immunosuppressive potency, with a capability to give rise to the production of immunoglobulines or other immunologically active substances (endotoxines, cytokines, lymphokines, prostaglandines, leucotrienes, other immuno modulators or biological messengers), including vaccines. Antibodies against any of these substances can also be used; preferred are immunotransfersomes with or without endotoxines, cytokines, prostaglandines, leucotrienes, with other immunomodulators, immunologically active cellular or molecular fragments, as well as corresponding antagonists, derivatives or precursors; particularly preferred compounds are lipid A and other glycolipids, muraminic acid derivatives, trehalose derivatives, phythaemaglutinines, lectins, polyinosine, polycytidylic acid (poli I:C), dimepranol-4-acetamidobenzoate, erythropoietin, 'granulocyte-macrophage colony stimulating factor' (GM-CSF), interleukine I and II, III and VI, interferon alpha, beta and/or gamma, leucotriene A, B, C, D, E and F, propandiamine, prostaglandine A, B, C, D, E, F, and I (prostacycline), tumor necrosis factor-alpha (TNF-alpha), thromboxan B, as well as immunoglobulines of types IgA, IgE, IgD, IgG, IgM; furthermore, suitable tissue and plant extracts, their chemical, biochemical or biological derivatives or replacements, their parts, such as characteristic peptide chains, etc.; as immunosuppressives, ganciclovir, azathiiprin, cyclosporin, FK 506 etc. are frequently used;

- at least one contraceptive agent, such as medroxyprogesteronacetate, lynesterol, lvonorgestrel, norethisteron, etc.;
- at least one circulation analeptic, such as cafedrin, etamivan, etilefrin, norfenefrin, pholedrin, theodrenalin, etc.;
- at least one drug for the therapy of liver diseases, such as orazamide, silymarin, or tiopromin;
- at least one substance with a light-protective function, such as mexenone;
- at least one antimalaria agent, such as amodiaquin, hydroxychloroquin or mepacrin;
- at least one substance for migraine or schizophrenia treatment, such as certain analeptics, beta-blockers, clonidin, dimetotiazine, ergotamine, lisurid (hydrogen maleate), methysergide, pizotifen, propranolol, proxibarbal, etc. Even more suitable are the serotonin antagonists or the blockers of serotonin receptors, such as 5-HT₁, 5-HT₂ or 5-HT₃; well suited for use according to this invention are also the receptor blockers AH21467 (Glaxo), AH25086 (Glaxo), GR43175 (Glaxo), GR38032 (Glaxo, = ondansetron), 5-hydroxytryptamine, ketanserine, methiothepin, alpha-methyl-5HT, 2-methyl-5HT, etc.;
- at least one mineral corticoid, such as aldosterone, fludrocortison, desoxycortonacetate, corresponding derivatives, etc.;
- at least one morphine antagonist (such as amiphenazol, lealvallorphan, nalorphine) or some substance with

morphine-like properties such as casomorphine, cyclo(leu-gly), dermorphine, met-enkephaline, methorphanide (tyr-gly-gly-phe-met-arg-arg-val), morphiceptine, morphine modulating neuropeptide (ala-gly-glu-gly-leu-ser-ser-pro-phe-trp-ser-leu-ala-ala-pro-gln-arg-phe-NH₂) etc.;

- at least one muscle relaxant, which frequently belongs to the groups of competitively or depolarising curare-agents, myotonolytics or analgetics; suitable substances with the desired effect are, among other materials, acetylsalicylic acid, alcuronium-chloride, azapropazon, atracuriumbesilate, baclofen, carisoprodol, quinine derivatives, chloromezanon, chlorophenesincarbamate, chlorozoxazon, dantrolen, decamethoniumbromide, dimethyltubocurariniumchloride, fenylamidol, gallamintriethiodide, guaiphensine, hexafluorenum-bromide, hexacarbacholinbromide, memantin, mephenesin, meprobamate, metamisol, metaxalon, methocarbamol, orphenadrin, paracetamol, phenazon, phenprobamate, suxamethoniumchloride, tetrazepam, tizanidin, tubocurarinchloride, tybamate, etc.;
- at least one narcotic, such as alfentanil, codeine, droperidol, etomidate, fentanil, flunitrazepam, hydroxybutiric acid, ketamine, methohexital, midazolam, thebacon, thiamylal, thiopental, etc., as well as corresponding derivatives;
- at least one substance with a neurotherapeutic activity, such as anaesthetics and vitamins, atropine-derivatives, benfotiamine, choline-derivatives, caffeine, cyanocobolamine, alpha-liponic acid, mepivacaine, phenobarbital, scopolamine, thiaminchloride hydrochloride, etc., and, most notably, procaine;

- at least one neuroleptic, e.g. butyrophenon-derivatives, phenotiazin-derivatives, tricyclic neuroleptics, as well as acetophenazine, benperidol, butaperazine, carfenazine, chloropromazine, chloroprothixen, clopenthixol, clozapine, dixyrazine, droperidol, fluanison, flupentixol, fluphenazine, fluspirilen, haloperidol, homofenazine, levomepromazine, melperon, moperon, oxipertin, pecazine, penfluridol, periciazine, perphenazine, pimozide, pipamperon, piperacetazine, profenamine, promazine, prothipendyl, sulforidazine, thiopropazate, thioproperazine, thioridazine, tiotixen, trifluoperazine, trifluperidol, triflupromazine, etc.; in particular, haloperidol and sulperide are often used for this purpose;
- at least one neurotransmitter or one of its antagonists; preferably, acetylcholine, adrenaline, curare (and, e.g. its antagonist edrophonium-chloride), dopamine, ephedrine, noradrenaline, serotonin, strychnine, vasotoline, tubocurarine, yohimbine, etc. are used;
- at least one opthalmic, in many cases from the groups of anaesthetics, antibiotics, corticoids, eye-tonics, chemotherapeutics, glaucoma agents, virustatics, antiallergics, vasodilators, or vitamins;
- at least one parasympathicomimetic (e.g. bethanechol-chloride, carbachol, demecarium-bromide, distigmin-bromide, pyridostigmin-bromide, scopolamine) or at least one parasympathicolytic (such as benzatropine, methscopolamine-bromide, pilocarpine or tropicamide);
- at least one agent for the therapy of psoriasis and/or neurodermitis; particularly well suited for this purpose are carrier substances with a hypoallergic action or the corresponding edge active compounds, with n-3 (omega 3),

less frequently with n-6 (omega 6), mainly with multiple, often 3-6, double bonds and/or hydroxy, more seldom methyl-, or oxo-side groups; these can also appear as side chains on further agent molecules; side groups on the 15th carbon atom are particularly efficient; as additives, amongst other substances, antimycotics, cytostatics, immunosuppressants or antibiotics can be used;

- at least one agent for the dilatation of the iris (mydriatic), such as atropine, atropinmethonitrate, cyclopentolate, pholedrine, scopolamine or tropicamide;
- at least one substance with a psychostimulating action; well suited for this purpose are, for example, amphetaminil, fencamfamine, fenetylline, meclofenoxate, methamphetamine, methylphenidate, pemoline, phendimetrazine, phenmetrazine, prolintane or viloxazine;
- at least one rhinologic, such as buphenine, cafaminol, carbinoxamide, chlorophenamin, chlorotenoxyzine, clemastine, dextromethorpane, etilefrine, naphazoline, norephedrine, oxymetazoline, phenylaprhine, piprinydrinate, pseudoephedrine, salicylamide, tramazoline, triprolidine, xylometazoline, etc.; from biological sources especially the radix gentiane extract;
- at least one somnifacient (such as sleep-inducing peptide (trp-ala-gly-gly-asg-ala-ser-gly-glu)), or a corresponding antagonist (such as bemegride);
- at least one sedative or tranquilizer, as the former, for example, acecarbromal, alimemazine, allobarbital, aprobarbital, benzoctamine, benzodiazepine-derivatives,

bromo-isoval, carbromal, chloropromazine, clomethiazol, diphenyl-methane-derivatives, estazolam, fenetylline, homofenazine, mebutamate, mesoridazine, methylpentynol, methylphenobarbital, molindone, oxomemazine, perazine, phenobarbital, promethazine, prothipendyl, scopolamine, secbutabarbital, trimetozine, etc.; as a tranquilizer, for example, azacyclonol, benactyzin, benzoctamine, benzquinamide, bromo-azepam, chlorodiazepoxide, chlorophenesincarbonat, clobazepam, diazepam, dipotassium-chloroazepate, doxepine, estazolam, hydroxyzine, lorazepam, medazepam, meprobamate, molindone, oxazepam, phenaglycodol, phenprobamate, prazepam, prochloroperazine, rescinnamine, reserpine or tybamate; drugs, such as distraneurine, hydantoine-derivatives, malonyl uric acid-derivatives (barbiturates), oxazolidine-derivatives, scopolamine, valepotriate, succinimide-derivatives, or hypnotics (e.g. diureides (such as barbiturates)), methaqualon, meprobamate, monoureides (such as carbromal), nitrazepam, or piperidin-dione, can be used for this purpose; amongst other substances, certain thymoleptics, such as librium or tofranil, can be used as antidepressants;

- at least one substance from the class of spasmolytics, e.g. adiphenine, alverine, ambicetamide, aminopromazine, atropine, atropine methonitrate, azintamide, bencyclane, benzarone, bevonium-methylsulfate, bietamiverine, butetamate, butylscopolammoniumbromide, camylofine, carzenide, chlorodiazepoxide, cionium-bromide, cyclandelate, cyclopentolate, dicycloverine, diisopromine, dimoxyline, diphemanil-methylsulfate, ethaverine, ethenzamide, fencarbamide, fenpipramide, fenpivnum-bromide, gefarnate, glycopyrroniumbromide, hexahydroadiphenin, hexocycliummethylsulfate, hymecromon,

isometheptene, isopropamidiodide, levomethadone, mebeverine, metamizon, methscopolamine-bromide, metixen, octatropine-methylbromide, oxazepam, oxybutin, oxyphenonium-bromide, papavorine, paracetamol, pentapiperide, penthienate-methobromide, pethidine, pipenzolate-bromide, piperidolate, pipoxolane, propanthelin-bromide, propylphenazon, propyromazine-bromide, racefemine, scopolamine, sulpiride, tiemonium-iodide, tridihexethyl iodide, tropenzilinbromide, tropinbenzilate, trospiumchloride, valetamathbromide, etc.; furthermore, belladonna alkaloids, papaverine and its derivatives, etc.;

- at least one sympathicolytic, e.g. azapetine or phentolamine;
- at least one sympathicomimetic, e.g. bamethane, buphenine, cyclopentamine, dopamine, L-(-)-ephedrine, epinephrine, etilefrine, heptaminol, isoetarine, metaraminol, methamphetamine, methoxamine, norfenefrine, phenylpropanolamine, pholedrine, propylhexedrine, protokylol or synephrine;
- at least one tuberculostatic, such as an antibiotic, p-aminosalicylic acid, capreomycine, cycloserine, dapson, ethambutol, glyconiazide, iproniazide, isoniazide, nicotinamide, protionamide, pyrarinamide, pyrodoxine, terizidone, etc., and, particularly preferred thereof, ethambitol and isoniazide;
- at least one urologic, e.g. a bladder tension modifying agent (such as cholinecitrate, distigminebromide, yohimbine), a corresponding antiinfection agents (antibiotics, chemotherapeutics, or nitrofurantoid-, chinolone-, or sulfonamide-derivative); furthermore,

2067754

- 59 -

adipinic acid, methionine, methenamine-derivatives, etc.;

- at least one substance with a vasoconstricting action; often, adrenalone, epinephrine, felypressine, methoxamine, naphazoline, oxymetazoline, tetrazyoline, tramazoline or xylometazoline are used for this purpose;
- at least one substance which is a vasodilator, such as e.g. azapetine, banethane, bencyclane, benfurodil-hemisuccinate, buphenine, butalamine, cinnarizine, diprophylline, hexyltheobromine, ifenprodil, isoxsuprine, moxisylyte, naftidrofuryl, nicotinyalcohol, papaverine, phenoxybenzamine, piribedil, primaperone, tolazoline, trimetazidine, vincamine or xantinol-nicotinate;
- at least one veins agent, e.g. aescine, benzarone, calcium-dobesilate, dihydroergotaminemesilate, diosmine, hyhydroxyethylrutoside, pignogenol, rutoside-aesinate, tribenoside, troxerutine, etc.;
- at least one virustatic, e.g. one immunostimulating agent, and/or an additional drug, such as as moroxydine or tromantadine, which may stimulate action of the immunostimulator;
- one agent for the treatment of wounds; for example, dexpanthenol, growth stimulating factors, enzymes or hormones, especially in combination with carriers which contain essential substances; povidon-iodide, fatty acids which are not straight, cetylpyridiniumchloride, chinoline-derivatives of known antibiotics and analgetics are useful;
- at least one substance with a toxic action or a toxin; common toxins from plant or microbial sources in

particular 15-acetoxyscirpenol, 3-acetyldeoxynivalenol, 3-alpha-acetyldiacetoxyscirpenol, acetyl T-2 toxin, aflatoxicol I, aflatoxicol II, aflatoxin B1, aflatoxin B2, aflatoxin B2-alpha, aflatoxin G1, aflatoxin G2, aflatoxin G2-alpha, aflatoxin M1, aflatoxin M2, aflatoxin P1, aflatoxin Q1, alternariol-monomethyl ether, aurovertin B, botulinum toxin D, cholera toxin, citreoviridin, citrinin, cyclopiazonic acid, cytochalasin A, cytochalasin B, cytochalasin C, cyrochalasin D, cytochalasin, cytochalasin H, cytochalasin J, deoxynivalenol, diacetoxyscirpenol, 4,15-diacetylverrucarol, dihydrocytochalasin B, enterotoxin STA, fusarenon X, iso T-2 toxin, O- methylsterigmatocystin, moniliformin, monoacetoxyscirpenol, neosolaniol, ochratoxin A, patulin, penicilinic acid, pertussis toxin, picrotoxin, PR-toxin, prymnesin, radicinin, roridin A, rubratoxin B, scirpentriol, secalonic acid D, staphylococcal enterotoxin B, sterigmatocystin, streptolysin O, streptolysin S, tentoxin, tetrahydrodeoxyaflatoxin B1, toxin A, toxin II, HT-2 toxin, T-2-tetraol, T-2 toxin, trichothecin, trichothecolon, T-2 triol, verrucaric acid, verrucarol, vomitoxin, zearalenol and zearalenon.

- at least one substance which affects growth in humans or animals, such as basic fibroblast growth factor (BFGF), endothelial cell growth factor (ECGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin, insulin-like growth factor I (LGF I), insulin-like growth factor II (LGF II), nerves-growth factor-beta (NGF-beta), nerves growth-factor 2,5s (NGF 2,5s), nerves growth-factor 7s (NGF 7s), platelet-derived growth factor (PDGF), etc.;
- a carrier and/or agent which creates a protective layer on and/or in a barrier, such as skin, against poison,

light UV-, gamma- or other radiation; against detrimental biological agents such as viruses, bacteria, toxins, etc.; carrier components and/or agents can hamper the detrimental action by chemical, biochemical, or biological means or else may prevent or diminish the penetration of such adversary agents;

- at least one fungicide, herbicide, pesticide, or insecticide;
- at least one plant hormone, e.g. abscisic acid, abscisic acid-methylester, 3-acetyl-4-thiazolidine-carboxyl acid, 1-allyl-1-(3,7-dimethyloctyl)-piperidinium bromide, 6-benzylaminopurine, 6-benzylaminopurine 9-(beta-glucoside), butanedio acid mono(2,2-dimethyl hydrazide), chlorocholine chloride, 2-chloroethyl-tris-(2'-methoxyethoxy)silane, 2-(o-chlorineophenoxy)-2-methylpropionic acid, 2-(p-chlorophenoxy)-2-methylpropionic acid, 2-(o-chlorophenoxy)propionic acid, 2-(m-chlorophenoxy)propionic acid, clofibrinic acid, colchicine, o-coumarinic acid, p-coumarinic acid, cycloheximide, alpha,beta-dichloroisobutiric acid, 2-(2,4-dichlorophenoxy)propanoic acid, 2,3-dihydro-5,6-diphenyl 1,4-oxathiine, dihydrozeatine, 6-(gamma,gamma-dimethylallylamino)purino riboside, 3-(2-[3,5-dimethyl-2-oxocyclohexyl-2-hydroxyethyl])-glutarimide, trans-2-dodecenedioic acid, ethyl-8-chloro-1-indazol-3-yl-acetate, N6-furfuryladenosine, 6-furfurylamino-purineriboside, gibberellic acid methylester, gibberellin A3-acetate, gibberellin A1 methylester, gibberellin A4 methylester, gibberellin A5 methylester, gibberellin A7 methylester, gibberellin A9 methylester, gibberellin A3 methylester 3,13-diacetate gibberinic acid, allo-gibberinic acid, gibberinic acid methylester, glyoxim, 22(s),23(s)-homobrassinolide, 9-hydroxyfluorene 9-

carboxylate, indol-3-acetic acid, indol-3-acetic acid ethylester, indol-3-propanoic acid, N6-(2-isopentenyl)adenine, N6-(2-isopentenyl)adenosine, 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylat methylchloride, kinetinglucoside, kinetinriboside, melissylalcohol, 1-methyladenine, methyl 2-chloro-9-hydroxy-fluorene-9-carboxylate, methyl 3,6-dichloro-o-anisate, 6-methylmercaptapurine, 1-naphthylacetamide, nonanoic acid methylester, 6-piperidino-1-purine, n-triacontanol, (-)-xanthoxine, zeatine glucosides, etc.;

- at least one pheromone or one pheromone-like substance, such as (-)-bornyl acetate, trans-5-decenol, cis-5-decenyl acetate, trans-5-decenyl acetate, 2,6-dichlorophenol, 1,7-dioxaspiro[5.5]undecane, trans-8,trans-10-dodecadienol ([E,E]-8,10-DDDOL), trans-7, cis-9-dodecadienyl acetate ([E,Z]-7,9-DDDA), trans-8, trans-10-dodecadienyl acetate ([E,E]-8,10-DDDA), cis-7-dodecen-1-ol (Z-7-DDOL), trans-10-dodecenol, cis-7-dodecenyl acetate (Z-7-DDA), cis-8-dodecenyl acetate, trans-8-dodecenyl acetate, 11-dodecenyl acetate, cis-7,8-epoxy-2-methyl-octadecane, cis-9-heneicosene, cis-7,cis-11-hexadecadienylacetate ([Z,Z]-7,11-HDDA), cis-7,trans-11-hexadecadienyl acetate ([Z,E]-7,11-HDDA), cis-9-hexadecenal (Z-9-HDAL), cis-11-hexadecenal (Z-11-HDAL), cis-11-hexadecenol (Z-11-HDOL), cis-11-hexadecenyl acetate (Z-11-HDA), trans-2-hexenyl acetate, cis-7-tetradecenal (Z-7-TDAL), cis-9-tetradecenol (Myristoleyl alcohol; Z-9-TDOL), cis-7-tetradecenol (Z-7-TDOL), cis-11-tetradecenol, cis-7-tetradecenyl acetate (Z-7-TDA), cis-9-tetradecenyl acetate (Myristoleyl acetate; Z-9-TDA), cis-11-tetradecenyl acetate (Z-11-TDA), trans-11-tetradecenyl acetate (E-11-TDA), cis-9-tetradecenyl formate

(Myristoleyl formate; Z-9-TDF), isoamyl acetate (acetic acid 3-methylbutyl ester), 2-methyl-3-buten-2-ol, 3-methyl-2-cyclohexen-1-ol, cis-14-methyl-8-hexadecenal, cis-2-methyl-7-octadecene, 4-methylpyrrole-2-carboxylic acid methyl ester (Methyl 4-methylpyrrole 2-carboxylate) cis-13-octadecenal 13-octadecyn-1-ol, 2-(phenyl)ethyl propionate (phenylethanol propanoate), propyl cyclohexylacetate, cis-9,trans-11-tetradecadienol ([Z,E]-9,11-TDDOL), cis-9,trans-11-tetradecadienyl acetate ([Z,E]-9,11-TDDA), cis-9,trans-12-tetradecadienyl acetate ([Z,E]-9,12-TDDA), trichloroacetic acid esters, cis-9-tricosene, undecanal, etc.;

- at least one pigment or one colouring substance;
- at least one carbohydrate;

A carbohydrate, normally, has a basic formula $C_x(H_2O)_y$, e.g. in sugar, starch, cellulose, and, moreover, can be derivatised in many different ways.

A monomeric carbohydrate residue is, for example, a natural monosaccharide residue, which in many cases is an adduct of a pentose or a hexose in aldose or ketose form which, in principle, can adopt L- or D-configurations. Owing to the space constraints and due to their greater biological relevance, only the latter will be referred to in the following.

An aldose with five carbon atoms (aldo-pentose, or simply pentose) is for example D-arabinose, D-lyxose, D-ribose or D-xylose.

A ketose with five carbon atoms (keto-pentose) is e.g. D-ribulose or D-xylulose.

An aldose with six carbon atoms (aldo-hexose, or simply hexose) is e.g. D-allose, D-altrose, D-galactose, D-glucose, D-mannose or D-talose. A ketose with six carbon atoms (or simply keto-hexose) is e.g. D-fructose, D-psicose, D-sorbose or D-tagatose.

A hexose, very frequently, exists in a cyclic form, as a pyranose (aldose), for example; alpha- or beta-D-glucopyranose are two typical examples for this. Another type of hexose is furanose, e.g. in an alpha- or beta-D-fructose. The pyranosyl residue is particularly preferably conjugated to a hydroxy group, the latter then being located in 1- or 6-positions; the furanosyl residue is preferably conjugated to the corresponding groups in positions 1- or 5-.

A carbohydrate residue, moreover, can be a natural disaccharide residue, e.g. a disaccharide residue consisting of two hexoses. Such a disaccharide residue arises, for example, through condensation of two aldoses, e.g. D-galactose or D-glucose, or one aldose, e.g. D-glucose and one ketose, e.g. fructose; disaccharides formed from two aldoses, such as lactose or maltose, are preferably conjugated to the phosphatidyl group through the hydroxy group, which is located in position 6- of the corresponding pyranosyl residue. A disaccharide formed from an aldose and a ketose, such as saccharose, is preferably conjugated through a hydroxyl-group in position 6- of the pyranosyl residue or in position 1- of the furanosyl residue.

A carbohydrate residue, moreover, is any derivatised mono-, di- or oligosaccharide residue, in which, for example, an aldehyde group and/or one or two terminal

hydroxy groups are oxidized to carboxy groups, e.g. in a D-glucar-, D-glucon- or D-glucuronic acid residue, all such residues being normally in the form of cyclic lactone residues. The aldehyde- or keto-groups in a derivatised mono- or disaccharide residue, moreover, can be reduced to hydroxy groups, e.g. in inositol, sorbitol or D-mannitol. Furthermore, individual hydroxy groups can be replaced by hydrogen atoms, e.g. in desoxysugars, such as 2-desoxy-D-ribose, L-fucose or L-rhamnose, or through amino groups, e.g. in aminosugars, such as D-galactosamine or D-glucosamine.

A carbohydrate can result from a cleaving action, starting with one of the mentioned mono- or disaccharides, by a strong oxidation agent, such as periodic acid. Amongst the biologically most important or most active carbohydrates are e.g. 2-acetamido-N-(epsilon-amino-caproyl)-2-deoxy-beta-glucopyranosylamine, 2-acetamido-1-amino-1,2-dideoxy-beta-glucopyranose, 2-acetamido-1-beta-(aspartamido)-1,2-dideoxyglucose, 2-acetamido-4,6-o-benzyliden-2-deoxy-beta-glucopyranose, 2-acetamido-2-deoxyallose, 3-acetamido-3-deoxyallose, 2-acetamido-2-deoxy-3-o-(beta-galactopyranosyl)-galactopyranose, 2-acetamido-2-deoxy-4-o-([4-o-beta-galactopyranosyl-beta-galactopyranosyl]-beta-galactopyranosyl)-glucopyranose, 2-acetamido-2-deoxy-3-o-(beta-galactopyranosyl)-alpha-glucopyranose, 6-o-(2-acetamido-2-deoxy-4-o-[beta-galactopyranosyl]-beta-glucopyranosyl)-galactopyranose, 4-o-acetamido-2-deoxy-6-o-(beta-galacto-4-o-(6-o-[2-acetamido-2-deoxy-beta-glucopyranosyl]-beta-galactopyranosyl) glucopyranose, 2-acetamido-2-deoxygalactose, 2-acetamido-2-deoxyglucose, 3-acetamido-3-deoxyglucose pyranose, 6-o-(2-acetamido-2-deoxy-beta-glucopyranosyl)-galactopyranose, 2-acetamido-2-deoxy-1-thio-beta-glucopyranose 3,4,6-

triacetate, acetopyruvic acid, N-acetylchondrosamine, N-acetylgalactosamine, N-acetylglucosamine, N-acetyl-alpha-glucosamine 1-phosphate, N-acetylglucosamine 6-phosphate, N-acetylglucosamine 3-sulfate, N-acetylglucosamine 6-sulfate, N-acetylheparine, N-acetyllactosamine, N-acetyl-beta-mannosamine, N-acetylneuraminic acid, N-acetylneuramine-lactose, 1-o-acetyl-2,3,5-tri-o-benzoyl-beta-ribofuranose, trans-aconic acid, adenine-9-beta-arabinofuranoside, adenosine 5'-diphosphoglucose, adenosine 5'-diphosphomannose, adonite, adonitol, adonose, agar, algin, alginic acid, beta-allose, alpha glycerophosphate, alpha ketoglutaric acid, altrose, (-)-altrose, p-aminobenzyl-1-thio-2-acetamido-2-deoxy-beta-glucopyranoside, N-epsilon-aminocaproyl-beta-fucopyranosylamine, N-epsilon-aminocaproyl-alpha-galactopyranosylamine, 2-amino-2-deoxygalactopyranose, 6-amino-6-deoxyglucopyranose, 1-amino-1-deoxy-beta-glucose, 6-aminoethyl-N-acetyl-beta-thioglucoaminide, 6-aminoethyl-1-thio-beta-galactopyranoside, 5-aminoimidazole-4-carboxamidoxime-1-beta-ribofuranosyl 3':5'-cyclo-monophosphate, delta-aminolevulinic acid, p-aminophenyl-2-acetamido-2-deoxy-beta-glucopyranoside, p-aminophenyl-2-acetamido-2-deoxy-1-thio-beta-glucopyranoside, p-aminophenyl-alpha-fucopyranoside, p-aminophenyl-alpha-galactopyranoside, p-aminophenyl-beta-galactopyranoside, p-aminophenyl-alpha-glucopyranoside, p-aminophenyl-beta-glucopyranoside, c-aminophenyl-beta-glucuronide, p-aminophenyl-1-thio-beta-glucuronide, p-aminophenyl-beta-lactopyranoside, p-aminophenyl-alpha-mannopyranoside, p-aminophenyl-beta-thiofucopyranoside, p-aminophenyl-1-thio-beta-galactopyranoside, p-aminophenyl-1-thio-beta-glucopyranoside, p-aminophenyl-1-thio-beta-xylopyranoside, p-aminophenyl-beta-xylopyranoside, 5-amino-1-(beta-ribofuranosyl)imidazole 4-carboxamide, amygdaline, n-amyl-beta-glucopyranoside, amylopectine, amylose, apigenine 7-

2067754

- 67 -

o-hesperidoside, arabinitol, arabinocytidine, 9-beta-arabinofuranosyladenine, 1-beta-arabinofuranosylcytosin, arabinose, arabinose 5-phosphate, arabinosylcytosine, arabite, arabitol, arbutine, atp-ribose, atractyloside, aurothiogluucose, n-butyl 4-o-beta-galactopyranosyl-beta-glucopyranoside, calcium gluconate, calcium heptagluconate, carboxyatractyloside, carboxymethylamylose, carboxymethylcellulose, carboxyethylthioethyl-2-acetamido-2-deoxy-4-o-beta-galactopyransol-beta-glucopyranoside, carboxyethylthioethyl 4-o-(4-o-[6-o-alpha-glucopyranosyl-alpha-glucopyranosyl]-alpha-glucopyranosyl)-beta-glucopyranoside, 4-o-(4-o-[6-o-beta-D-galactopyranosyl-beta-D-galactopyranosyl]-D-glucopyranose, carrageenan, D(+)cellobiose, D(+)cellopentaose, D(+)cellotetraose, D(+)cellotriose, cellulose, cellulose caprate, cellulose carbonate, chitin, chitobiose, chitosan, chitotriose, alpha-chloroalose, beta-chloroalose, 6-chloro-6-deoxy-alpha-glucopyranose, chondroitin sulfate, chondrosamine, chondrosine, chrysophanic acid, colominic acid, convallatoxin, alpha-cyclodextrine, beta-cyclodextrine, cytidine 5'-diphosphoglucose, cytosine 1-beta-arabinofuranoside, daunosamine, n-decyl-beta-glucopyranoside, 5-deoxyarabinose, 2-deoxy-2-fluoroglucose, 3-deoxy-3-fluoroglucose, 4-deoxy-4-fluoroglucose, 6-deoxygalacto pyranose, 2-deoxygalactose, 1-deoxyglucohex-1-eno-pyranose tetrabenzoat, 2-deoxyglucose, 6-deoxyglucose, 2-deoxyglucose 6-phosphate, 1-deoxymannojerimycin, 6-deoxymannose, 1-deoxy-1-morpholinofructose, 1-deoxy-1-nitroalutol, 1-deoxy-1-nitroaltitol, 1-deoxy-1-nitrogallactitol, 1-deoxy-1-nitromannitol, 1-deoxy-1-nitrosorbitol, 1-deoxy-1-nitrotalitol, deoxynojirimycine, 3-deoxy-erythro-pentose, 2-deoxy-6-phosphogluconic acid, 2-deoxyribose, 3-deoxyribose, 2-deoxy-alpha-ribose 1-

2067754

- 68 -

phosphate, 2-deoxyribose 5-phosphate, 5-deoxyxylofuranose, dextran, dextransulfate, dextrine, dextrose, diacetonefructose, diacetone mannitol, 3,4-di-o-acetyl-6-deoxyglucal, di-o-acetyl rhamnal, 2,3-diamino-2,3-dideoxy-alpha-glucose, 6,9-diamino-2-ethoxyacridine lactate, 1,3:4,6-di-o-benzylidene mannitol, 6,6'-dideoxy-6,6'-difluorotrehalose, digalactosyl diglyceride, digalacturonic acid, (+)digitoxose, 6,7-dihydrocoumarin-9-glucoside, dihydroxyacetone, dihydroxyacetone phosphate, dihydroxyfumaric acid, dihydroxymalic acid, dihydroxytartaric acid, dihydrozeatinriboside, 2,3-diphosphoglycerolic acid, dithioerythritol, dithiothreitol, n-dodecyl beta-glucopyranoside, n-dodecyl beta-maltoside, dulcitol, elemi-gum, endotoxin, epifucose, erythritol, erythro-pentulose, erythrose, erythrose 4-phosphate, erythrulose, esculin, 17-beta-estradiol-3-glucuronide 17-sulfate, estriole glucuronide, estron beta-glucuronide, ethodin, ethyl 4-o-beta-D-galactopyranosyl)-beta-D-glucopyranoside, ethyl 2-acetamido-4-o-(2-acetamido-2-deoxy-beta-glucopyranosyl)-6-o-(alpha-fucopyranosyl)-2-deoxy-beta-glucopyranoside, ethyl 2-acetamido-2-deoxy-4-o-(4-o-alpha-galactopyranosyl-beta-galactopyranosyl)-beta-glucopyranoside, ethyl cellulose ethylene glycol chitin, ethyl 4-o-(4-o-alpha-galacto-pyranosyl-beta-galactopyranosyl)-beta-glucopyranoside, ethyl 4-o-beta-galactopyranosyl-beta-glucopyranoside, ethyl pyruvate, ethyl beta-thiogluconide, etiocholane-3alpha-ol-17-on glucuronide, ficoll, 6-fluoro-6-deoxyglucose, franguloside, fraxin, fructosazine, beta-(-)fructose, fructose-1,6- diphosphate, fructose-2,6-diphosphate, fructose-1-phosphate, fructose-6-phosphate, fucoidan, fucose, alpha -(-)-fucose-1-phosphate, fucosylamine, 2'-fucosyllactose, 3-fucosyllactose, fumaric acid, galactal,

2067754

- 69 -

galactitol, galactopyranosylamine, 3-o-beta-galactopyranosyl-arabinose, 4-o-beta-galactopyranosyl-fructofuranose, 4-o-(4-o-beta-galactopyranosyl beta-galactopyranosyl)-glucopyranose, 4-o-alpha-galactopyranosyl-galactopyranose, 6-o-beta-galactopyranosylgalactose, 4-o-(beta-galactopyranosyl)-alpha-mannopyranose, alpha-galactopyranosyl 1-phosphate, galactopyranosyl-beta-thio-galactopyranoside, (+)galactosamine, alpha-galactosamine 1-phosphate, alpha-galactose 1-phosphate, galactose 6-phosphate, galactose 6-sulfate, 6-(alpha-galactosido)glucose, galacturonic acid, beta-gentiobiose, glucan, glucitol, glucoheptonic acid, glucoheptose, glucoheptulose, gluconate 6-phosphate, gluconic acid, 1-o-alpha-glucopyranosyl-beta-fructofuranoside, 6-o-alpha-glucopyranosylfructose, 1-o-alpha-glucopyranosyl-alpha-glucopyranoside, 4-o-beta-glucopyranosylglucopyranose, 4-o-(4-o-[6-o-alpha-glucopyranosyl-alpha-glucopyranosyl]-alpha-glucopyranosyl) glucopyranose, (+)glucosamine, alpha-glucosamine 6-2,3-disulfate, alpha-glucosamine 1-phosphate, glucosamine 6-phosphate, glucosamine 2-sulfate, alpha-glucosamine 3-sulfate, glucosamine 6-sulfate, glucosaminic acid, glucose, alpha-glucose 1,6-diphosphate, glucose 1-phosphate, glucose 6-phosphate, glucose 6-sulfate, glucuronamide, glucuronic acid, alpha-glucuronic acid 1-phosphate, glyceraldehyde, glyceraldehyde 3-phosphate, glycerate 2,3-diphosphate, glycerate 3-phosphate, glyceralic acid, alpha-glycerophosphate, beta-glycerophosphate, glycogen, glycolaldehyde, glycol chitosan, n-glycolylneuraminic acid, glycyric acid, glyoxylic acid, guanosine, 5'-diphosphoglucose, gulose, gums (accroides, agar, arab, carrageenan, damar, elemi, ghatti, guaiac, guar, karaya, locust bonne, mast, pontianac, storax, tragacanth, xanthan), heparin and heparin-like substances

2067754

- 70 -

(mesoglycan, sulodexide, etc.), heptakis (2,3,6-tri-o-methyl)-beta-cyclodextrin, heptanoyl-N-methylglucamide, n-heptyl beta-glucopyranoside, hesperidin, n-hexyl-beta-glucopyranoside, hyaluronic acid, 16-alpha-hydroxyestronglucuronide, 16-beta-hydroxyestron glucuronide, hydroxyethyl starch, hydroxypropylmethyl-cellulose, 8-hydroxyquinolin-beta-glucopyranoside, 8-hydroxyquinolin glucuronide, idose, (-)-idose, indole-3-lactic acid, indoxyl-beta-glucoside, epi-inositol, myo-inositol, myo-inositol bisphosphate, myo-inositol-1,2-cyl phosphate, scyllo-inositol, inositolhexaphosphate, inositolhexasulfate, myo-insoitol 2-monophosphate, myo-inositol trisphosphate, (q)-epi-inosose-2, scyllo-inosose, inulin, isomaltose, isomaltotriose, isosorbid dinitrate, 11-ketoandrosterone beta-glucuronide, 2-ketogluconic acid, 5-ketogluconic acid, alpha-ketopropionic acid, lactal, lactic acid, lactitol, lactobionic acid, lacto-N-tetraose, lactose, alpha-lactose 1-phosphate, lactulose, laminaribiose, laminnarine, levoglucosan, beta-levulose, lichenan, linamarine, lipopolysaccharides, lithiumlactate, lividomycine A, lyxose, lyxosylamine, maltitol, maltoheptaose, maltohexaose, maltooligosaccharide, maltopentaose, maltose, alpha-(+)maltose 1-phosphate, maltotetraose, maltotriose, malvidine-3,5-diglucoside, mandelonitril beta-glucoside, mandelonitril glucuronic acid, mannan, mannit, mannitol, mannitol 1-phosphate, alpha-mannoheptitol, mannoheptulose, 3-o-alpha-mannopyranosyl-mannopyranose, alpha(+)mannopyranosyl-1-phosphate, mannosamine, mannosan, mannose, A(+)mannose 1-phosphate, mannose 6-phosphate, (+)melezitose, A(+)melibiose, mentholglucuronic acid, 2-(3'-methoxyphenyl)-N-acetylneuraminic acid, methyl 3-o-(2-acetamido-2-deoxy-beta-galaotopyranosyl)-alpha-

galactopyranoside, methyl 4-o-(3-o-[2-acetamido-2-deoxy-4-o-beta-galactopyranosyl beta-glucopyranosyl]-beta-galactopyranosyl)-beta-glucopyranoside, methyl 2-acetamido-2-deoxy-beta-glucopyranoside, methyl 3-o-(2-acetamido-2-deoxy-beta-glucopyranosyl)-beta-galactopyranoside, methyl 6-o-(2-acetamido)-2-deoxy-beta-glucopyranosyl)-alpha-mannopyranoside, methyl acosaminide, methyl alpha-altropyranoside, methyl 3-amino-3-deoxy-alpha-mannopyranoside, methyl beta-arabinopyranoside, methyl 4,6-o-benzylidene-2,3-di-o-toluenesulfonyl-alpha-galactopyranoside, methyl 4,6-o-benzylidene-2,3-di-o-p-toluenesulfonyl-alpha-glucopyranoside, methyl cellulose, methyl alpha-daunosaminide, methyl 6-deoxy-alpha-galactopyranoside, methyl 6-deoxy-beta-galactopyranoside, methyl 6-deoxy-alpha-glucopyranoside, methyl 6-deoxy-beta-glucopyranoside, methyl 3,6-di-o-(alpha-mannopyranosyl)-alpha-mannopyranoside, 1-o-methyl-alpha-galactopyranoside, 1-o-methyl-beta-galactopyranoside, methyl 3-o-alpha-galactopyranosyl-alpha-galactopyranoside, methyl-3-o-beta-galactopyranosyl-beta-galactopyranoside, 4-o-(2-o-methyl-beta-galactopyranosyl) glucopyranose, methyl 4-o-beta-galactopyranosyl-beta-glucopyranoside, methyl-4-o-(beta-galactopyranosyl-alpha-mannopyranoside, 5-5-methylgalactopyranose, methylgalactoside, n-methylglucamine, 3-o-methyl-alpha-glucopyranose, 1-o-methyl-alpha-glucopyranoside, 1-o-methyl-beta-glucopyranoside, alpha-methyl glucoside, beta-methyl glucoside, methyl glycol chitosan, methyl-alpha-mannopyranoside, methyl-2-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl 3-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl-4-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl 6-o-alpha-mannopyranosyl-alpha-mannopyranoside, methyl alpha-rhamnopyranoside, methyl alpha-ribofuranoside, methyl beta-ribofuranoside,

2067754

- 72 -

methylbeta-thiogalactoside, methyl 2,3,5-tri-o-benzoyl-alpha-arabinofuranoside, 4-methylumbelliferyl2-acetamido-4,6-o-benzylidene-2-deoxy-beta-glucopyranoside, 4-methylumbelliferyl N-acetyl-beta-galactosaminide, 4-methylumbelliferyl N-acetyl-alpha-glucosaminide, 4-methylumbelliferyl-N-acetyl-beta-glucosaminide, 4-methylumbelliferyl-alpha-arabinofuranoside, 4-methylumbelliferyl-alpha-arabinopyranoside, 4-methylumbelliferyl-beta-cellobioside, 4-methylumbelliferyl-beta-n,n'-diacetylchitobioside, 4-methylumbelliferyl alpha-fucoside, 4-methylumbelliferyl beta-fucoside, 4-methylumbelliferyl alpha-galactopyranoside, 4-methylumbelliferyl beta-galactopyranoside, 4-methylumbelliferyl alpha-galactoside, 4-methylumbelliferyl beta -glucopyranoside, 4-methylumbelliferyl alpha-glucoside, 4-methylumbelliferyl beta-glucoside, 4-methylumbelliferyl beta-glucuronide, 4-methylumbelliferyl beta-mannopyranoside, 4-methylumbelliferylbeta-n,n',n''-triacetylchitotriose, 4-methylumbelliferyl2,3,5-tri-o-benzyl-alpha-arabinofuranoside, 4-methylumbelliferyl beta-xyloside, methyl beta-xylopyranoside, 2-o-methylxylose, alpha-methylxyloside, beta-methylxyloside, metrizamide, 2'-monophosphoadenosine 5'-diphosphoribose, 2'-monophosphoinosine 5'-diphosphoribose, mucine, muraminic acid, naringine, sodium lactate, sodium polypectate, sodium pyruvate, neoagarobiose, neoagarohexaitol, neoagarohexaose, neoagarotetraose, beta-neocarrabiose, neocarrabiose 4/1-sulfate, neocarrahexaose(2/4,4/1,4/3,4/5)-tetrasulfate, neocarratetraose(4/1,4/3)-disulfate, neocarratetraose(4/1)-sulfate, neohesperidin, dihydrochalcon, neohesperidose, neuraminic acid, neuraminic acid beta-methylglycoside, neuramine-lactose, nigeran, nigerantetrasaccharide, nigerose, n-nonyl glucoside, n-nonylbeta-glucopyranoside, octadecylthioethyl 4-o-alpha-galactopyranosyl-beta-galactopyranoside,

octadecylthioethyl 4-o-(4-o-[6-o-alpha-glucopyranosyl-
 alpha-glucopyranosyl]-alpha-glucopyranosyl)-beta-
 glucopyranoside, octanoyl n-methylglucamide, n-octyl
 alpha-glucopyranoside, n-octyl-beta-glucopyranoside,
 oxidised starch, pachyman, palatinose, panose,
 pentaerythritol, pentaerythritol diformal, 1,2,3,4,5-
 pentahydroxy, capronic acid, pentosanpolysulfate,
 perseitol, phenolphthalein glucuronic acid,
 phenolphthalein mono-beta-glucosiduron phenyl 2-
 acetamido-2-deoxy-alpha-galactopyranoside, phenyl2-
 acetamido-2-deoxy-alpha- glucopyranoside, alpha-phenyl-
 N-acetyl-glucosaminide, beta-phenyl N-acetyl-
 glucosaminide, phenylethyl beta- galactoside, phenyl
 beta-galactopyranoside, phenyl beta-galactoside,
 phenyl alpha-glucopyranoside, phenyl beta-gluco-
 pyranoside, phenyl alpha-glucoside, phenyl beta-
 glucoside, phenyl beta-glucuronide, beta-phenyllactic
 acid, phenyl alpha-mannopyranoside, beta-phenylpyruvic
 acid, phenyl beta-thiogalactopyranoside, phenyl beta-
 thiogalactoside, phospho(enol)pyruvate, (+)2-
 phosphoglyceric acid, (-)3-phosphoglyceric acid,
 phosphohydroxypyruvic acid, 5-phosphorylribose 1-
 pyrophosphate, phytic acid, poly-N-acetylglucosamine,
 polygalacturonic acid, polygalacturonic acid methyl
 ester, polypectate, sodium, polysaccharide, 5beta-
 pregnane-3alpha,20alpha-diol glucuronide, n-propyl4-o-
 beta-galactopyranosyl-beta-glucopyranoside, prunasine,
 psicose, pullulan, quinolyl-8beta-glucuronic acid,
 (+)raffinose, alpha-rhamnose, rhapontine, ribitol,
 ribonolacton, ribose, D-2-ribose, alpha-ribose 1-
 phosphate, ribose 2-phosphate, ribose 3-phosphate, ribose
 5-phosphate, ribulose, ribulose-1,5-diphosphate, ribulose
 6-phosphate, saccharic acid, saccharolactic acid,
 saccharose, salicin, sarcolactic acid, schardingers-
 alpha-dextrine, schardingers-beta-dextrine,

sedoheptulosan, sedoheptulose 1,7-diphosphate, sialic acid, sialyllactose, sinigrine, sorbitol, sorbitol 6-phosphate, (+)-sorbitol, (-)-sorbitol, stachyose, starch, storax, styrax, sucrose, sucrose monocaprinate, tagatose, alpha-talose, (-)-talose, tartaric acid, testosterone-beta-glucuronide, 2,3,4,6-tetra-O-methyl-glucopyranose, thiodiglycoside, 1-thio-beta-galactopyranose, beta-thioglucofuranose, 5-thioglucofuranose, 5-thioglucofuranose 6-phosphate, threitol, threose, (+)-threose, (-)-threose, thymidine 5'-diphosphoglucose, thymine 1-beta-arabinofuranoside, tragacanth, (+)-trehalose, trifluorothymine, deoxyriboside, 3,3',5-trihydroxy-4'-methoxy-stilbene-3-O-beta-glucoside, trimethylsilyl(+)-arabinose, trimethylsilyldulcitol, trimethylsilyl-beta (-)-fructose, trimethylsilyl(+)-galactose, trimethylsilyl-alpha-(+)-glucose, trimethylsilyl(+)-mannitol, trimethylsilyl(+)-rhamnose, trimethylsilyl(-)-sorbitol, trimethylsilyl(+)-xylose, rac-1-O-tritylglycerol, (+)-turanose, n-undecyl beta-glucopyranoside, uracil beta-arabinofuranoside, uridine 5'-diphospho-N-acetylglucosamine, uridine 5'-diphosphogalactose, uridine 5'-diphosphoglucose, uridine 5'-diphosphoglucuronic acid, uridine 5'-diphosphomannose, uridine 5'-diphosphoxylose, vancomycin, xanthan gum, xylane, xylite, xylitol, xylobiose, alpha-xylopyranosyl 1-phosphate, xylose, alpha-xylose 1-phosphate, xylose 5-phosphate, xylotriose, xylulose, xylulose 5-phosphate, yacca, zeatine riboside, zinclactate, zymosan A, etc.

Designations desoxyribonucleic-(DNA) and ribonucleic acid (RNA) have their common meaning; preferably such DNA or RNA forms, or their antagonists, are used which have a particularly strong biological action.

- at least one nucleotide, peptide, protein or a related compound;

Nucleotides, which can be effectively transported with the aid of transfersomes, encompass adenine, adenosine, adenosine-3',5'-cyclic monophosphate, N6,02'-dibutyryl, adenosine-3',5'-cyclic monophosphate, N6,02'-dioctanoyl, adenosine, n6-cyclohexyl, salts of adenosine-5'-diphosphate, adenosine-5'-monophosphoric acid, adenosine-5'-o-(3-thiotriphosphate), salts of adenosine-5'-triphosphate, 9-beta-D-arabinoturanosyladenine, 1-beta-D-arabinoturanosylcytosine, 9-beta-D-arabinoturanosylguanine, 9-beta-D-arabinoturanosylguanine 5'-triphosphate, 1-beta-D-arabinoturanosylthymine, 5-azacytidine, 8-azaguanine, 3'-azido-3'-deoxythymidine, 6-benylaminopurine, cytidine phosphoramidite, beta-cyanoethyl diisopropyl, 249802cytidine-5'-triphosphate, 2'-deoxyadenosine, 2'-deoxyadenosine 5'-triphosphate, 2'-deoxycytidine, 2'-deoxycytidine 5'-triphosphate, 2'-deoxyguanosine, 2'-deoxyguanosine 5'-triphosphate, 2',3'-dideoxyadenosine, 2',3'-dideoxyadenosine 5'-triphosphate, 2',3'-dideoxycytidine, 2',3'-dideoxycytidine 5'-triphosphate, 2',3'-dideoxyguanosine, 2',3'-dideoxyguanosine 5'-triphosphate, 2',3'-dideoxyinosine, 2',3' dideoxythymidine, 2',3'-dideoxythymidine 5'-triphosphate, 2',3'-dideoxyuridine, N6-dimethylallyl-adenine, 5-fluoro-2'-deoxyuridine, 5-fluorouracil, 5-fluorouridin, 5-fluorouridine 5'-monophosphate, formycin A 5'-triphosphate, formycin B, guanosine-3'-5'-cyclic monophosphate, guanosine-5'-diphosphate-3'-diphosphate, guanosine-5'-o-(2-thiotriphosphate), guanosine-5'-o-(3'-thiotriphosphate), guanosine 5'-triphosphate, 5'-guanylylimidodiphosphate, inosine, 5-iodo-2'-deoxyuridine, nicotinamide-adenine dinucleotides, nicotinamide-adenine dinucleotides, nicotinamide-adenine dinucleotide phosphate, oligodeoxythymidylic acid, (p(dT)10), oligodeoxythymidylic acid (p(dT)12-18), polyadenylic acid

(poly A), polyadenylic acid-oligodeoxythymidynic acid, polycytidylic acid, poly(deoxyadenyl-deoxythymidylic acid, polydeoxyadenylic-acid-oligodeoxythymidynic acid, polydeoxythymidylic acid, polyinosine acid-polycytidylic acid, polyuridynic acid, ribonucleic acid, tetrahydro-uridine, thymidine, thymidine-3',5'-diphosphate, thymidine phosphoramidite, beta-cyanoethyl diisopropyl, 606102 thymidine 5'-triphosphate, thymine, thymine riboside, uracil, uridine, uridine-5'-diphosphoglucose, uridine 5'-triphosphate, xanthine, zeatine, transeatine riboside, etc. Further suitable polymers are: poly(DA) ss, poly(A) ss, poly(C) ss, poly(G) ss, poly(U) ss, poly(DA)-(DT) ds, complementary homopolymers, poly (D(A-T)) ds, copolymers, poly(DG)-(DC) ds, complementary homopolymers, poly (d(G-C)) ds copolymers, poly (d(L-C)) ds copolymers, poly(I)-poly(C) ds, etc. An oligopeptide or a polypeptide preferably contains 3-250, frequently 4-100, and very often 4-50 amino acids which are mutually coupled via amide-bonds. Suitable amino acids are usually of the alpha- and L-type; exceptions, however, such as in dermorphine are possible.

Peptides with a particularly high biological and/or therapeutic significance, and which can also be combined with transfersomes, are, for example, N-acetyl-Ala-Ala-Ala-, N-acetyl-Ala-Ala-Ala methyl ester, N-acetyl-Ala-Ala-Ala-Ala, N-acetyl-Asp-Glu, N-acetyl-Gly-Leu, N-alpha-Acetyl-Gly-Lys methyl ester acetate, acetyl-hirudine fragments, acetyl-5-hydroxy-Trp-5-hydroxy-Trp amide, des-acetyl-alpha-melanocyte stimulating hormone, N-Acetyl-Met-Asp-Arg-Val-Leu-Ser-Arg-Tyr, N-acetyl-Met-Leu-Phe, acetyl-muramyl-Ala-isoGln, N-acetyl-Phe-Tyr, N-acetyl-Phe-norLeu-Arg-Phe amide, N-acetyl-renine substrate tetradecapeptide, N-acetyl-transforming growth factor, adipokinetic hormone II, adjuvant peptide,

adrenal peptide E, adrenocorticotrophic hormone (ACTH 1-39, Corticotropine A) and its fragments such as 1-4 (Ser-Tyr-Ser-Met), 1-10 (Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly), 1-17, 1-24 and 1-39, 11-24, 18-39, Ala-Ala, beta-Ala-Ala, Ala-Ala-Ala, Ala-Ala-Ala methyl ester, Ala-Ala-Ala-Ala, Ala-Ala-Ala-Ala-Ala, Ala-Ala-Ala-Ala-Ala-Ala, Ala-Ala-Phe, 7-amido-4-methylcoumarin, Ala-Ala-Phe p-nitroanilide, Ala-Ala-Val-Ala p-nitroanilide, Ala-Arg-Pro-Gly-Tyr-Leu-Ala-Phe-Pro-Arg-Met amide, beta-Ala-Arg-Ser-Ala-Pro-Thr-Pro-Met-Ser-Pro-Tyr, Ala-Asn, Ala-Asp, Ala-Glu, Ala-gamma-Gln-Lys-Ala-Ala, Ala-Gly, beta-Ala-Gly, Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Tyr-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe amide, Ala-Gly-Gly, Ala-Gly-Ser-Glu, Ala-His, beta-Ala-His, Ala-isoGln-Lys-Ala-Ala, Ala-Ile, Ala-Leu, beta-Ala-Leu, Ala-Leu-Ala, Ala-Leu-Ala-Leu, Ala-Leu-Gly, Ala-Lys, beta-Ala-Lys, Ala-Met, N-beta-Ala-1-methyl-His, Ala-norVal, Ala-Phe, beta-Ala-Phe, Ala-Phe-Lys 7-amido-4-methylcoumarin, Ala-Pro, Ala-Pro-Gly, Ala-sarcosine, Ala-Ser, Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr, Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr amide, Ala-Thr, Ala-Trp, beta-Ala-Trp, Ala-Tyr, Ala-Val, beta-Ala-Val, beta-Ala-Trp-Met-Asp-Phe amide, alytesine, amanitine, amastatine, angiotensine I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu), II II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), III and related peptides, angiotensine II antagonist, angiotensine II receptor binding protein, angiotensine converting enzyme and its inhibitor (e.g. entipaine, bestatine, chymostatine, E-64, elastatinal, etc.) anserine, antide, aprotinine, arginine, vasopressine-Ala-Gly, Arg-Ala, Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly, Arg-Asp, Arg-Glu, Arg-Gly, Arg-Gly-Asp, Arg-Gly-Asp-Ser, Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Lys-Pro, Arg-Gly-Glu-Ser, Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala, Arg-His-Phe, Arg-Ile, Arg-Leu, Arg-Lys, Arg-Lys-Asp-Val-Tyr, Arg-Phe, Arg-Phe-Asp-Ser, Arg-Pro-Pro-Gly-Phe-Ser

Pro-Phe-Arg, Arg-Ser-Arg, Arg-Ser-Arg-His-Phe, Arg-Val, Asn-Pro-Asn-Ala-Asn-Pro-Asn-Ala, Asn-Pro-Asn-Ala-Asn-Pro-Asn-Ala-Asn-Pro-Asn-Ala, alpha-Asp-Ala, Asp-Ala-Glu-Asn-Leu-Ile-Asp-Ser-Phe-Gln-Glu-Ile-Val, Asp-Asp, alpha-Asp-Glu, alpha-Asp-Gly, beta-Asp-Gly, beta-Asp-His, Asp-Leu amide, beta-Asp-Leu, alpha-Asp-Lys, alpha-Asp-Phe amide, alpha-Asp-Phe, alpha-Asp-Phe methyl ester, beta-Asp-Phe methyl ester, alpha-Asp-Ser-Asp-Pro-Arg, Asp-Val, beta-Asp-Val, atrial natriuretic peptide, especially its fragments 1-32 and 5-28, atriopeptine I, II and III, auriculine A and B, beauvericine, beniotript, bestatine, N-benzylated peptides, big gastrin I, bombesin, (D-Phe₁₂,Leu₁₄) (Tyr₄), (Lys₃)-bombesin, (Tyr₄)-bombesin, adrenal medulla docosa-peptide and dodeca-peptide, Bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and related peptides, Bradykinin potentiators, brain natriuretic peptide, buccaline, bursine, S-t-butyl-Cys, caeruleine, calcitonin, calcitonin gene related peptide I and II, calmodulin binding domain, N-carboxymethyl-Phe-Leu, N-((R,S)-2-carboxy-3-phenyl-propionyl)Leu, cardioactive peptides A and B, carnosine, beta-casomorphine, CD4, cerebelline, N-chloroacetyl-Gly-Gly, chemotactic peptides such as formylated substances, cholecystokinin fragments, e.g., cholecystokinin octapeptide, coherine etc.

Also worth mentioning are the collagen peptides, conicostatine, conicotropine releasing factor, conotoxin G1, M1, and GVIA, corticotropine-like intermediate lobe peptide, corticotropine releasing factor and related peptides, C-peptide, Tyr-C-peptide, cyclic calcitonin gene related peptides, cyclo(His-Phe-), cyclo(His-Pro-), cyclo(Leu-Gly-), cyclo(Pro-Gly-), Cys-Asp-Pro-Gly-Tyr-Ile-Ser-Arg amide, Cys-Gln-Asp-Ser-Glu-Thr-Arg-Thr-Phe-Tyr, DAGO, Delta-sleep inducing peptide, dermorphine,

(Ser(Ac)7)-dermorphine, diabetes associated peptide and its amide, N-alpha,N-epsilon-diacetyl-Lys-Ala-Ala, N-2,4-dinitrophenyl-Pro-Gln-Gly-Ile-Ia-Gly-Gln-Arg, diprotine A, dynorphines such as dynorphine A (Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Sn-Gln), fragments 1-6 (leucine enkephalin-Arg), 1-8, 1-13 or E-64, dynorphine B, ebelactones (e.g. A and B) ecarine, elastatinal, eledoisine and related peptides, alpha-, beta- und gamma-endorphine, endothelins, endorphines (e.g. alpha (=beta-Lipotropine 61-76), (Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr), beta (=beta-Lipotropine 61-91) and other beta-lipotropine-fragments, enkephalin and Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) and related peptides, enkephalinase inhibitors (e.g. epimastatine, epibestatine, foroxymithine, leupeptine, pepstatine, Nle-Sta-Ala-Sta), eosinophilotactic tetrapeptide, epimastatine, epibestatine, (Cys(Acm)20,31)-epidermal growth factor and its fragments or receptors, epidermal mitosis inhibiting pentapeptide, trans-epoxysuccinyl-Leu amido-(4-guanidino)butane, erythropoietine and fragment, S-ethylglutathione, fibrinogen related peptide, fibrinopeptide A and B, Tyr-fibrinopeptide A, (Glu1)-fibrinopeptide S, fibrinopeptide B-Tyr, fibroblast growth factor fragment 1-11, follicular gonadotropine releasing peptide, N-formylated peptides, foroxymithine, N-(3(2-furyl)acryloyl) peptide derivatives, galanine, GAP 1-13, gastric inhibitory polypeptide, gastrin related peptides and derivatives, gastrin releasing peptide, gastrointestinal peptides (e.g. Ala-Trp-Met-Asp-Phe-Amid, bombesin, caerulein, cholecystokinin, gelanin, gastrin, glucagon, motilin, neuropeptide K, pancreatic polypeptide, pancreozymin, Phi-27, secretin, valosin, etc.), Gln-Ala-Thr-Val-Gly-Asp-Val-Asn-Thr-Asp-Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys, (des-His1, Glu9)-glucagon amide, glucagon (1-37),

glucagon-like peptide I, alpha-Glu-Ala, Glu-Ala-Glu, Glu-Ala-Glu-Asn, alpha-Glu-Glu, gamma-Glu-Glu, gamma-Glu-Gln, gamma-Glu-Gly, PGlu-Gly-Arg-Phe amide, alpha-Glu-Gly-Phe, gamma-Glu-His, gamma-Glu-Leu, alphaGlu-alpha-Lys, gamma-Glu-epsilon-Lys, N-gamma-Glu-Phe, PGlu-Ser-Leu-Arg-Trp amide, alpha-Glu-Trp, gamma-Glu-Trp, gamma-Glu-Tyr, alpha-Glu-Val, gamma-Glu-Val, PGlu-Val-Asn-Phe-Ser-Pro-Gly-Trp-Gly-Thr amide, A-Glu-Val-Phe, glutathiones and related peptides, glutathionesulfonic acid, Gly-Ala, Gly-beta-Ala, Gly-Ala-Ala, Gly-Ala-Ala-Ala-Ala, Gly-Ala-Tyr, Gly-alpha-aminobutyric acid, Gly-gamma-aminobutyric acid, Gly-Arg-Ala-Asp-Ser-Pro-Lys, Gly-Arg-Ala-Asp-Ser-Pro-OH, Gly-Arg-Gly-Asp-Ser, Gly-Arg-Gly-Asp-Asn-Pro-OH, Gly-Arg-Gly-Asp-Ser-OH, Gly-Arg-Gly-Asp-Ser-Pro-Lys, Gly-Arg-Gly-Asp-Ser-Pro-OH, Gly-Arg-Gly-Asp-Thr-Pro, Gly-Arg-Gly-Asp-Thr-Pro-OH, Gly-Arg p-nitroanilide, Gly-Arg-Gly-Asp, Gly-Arg-Gly-Asp-Ser, Gly-Asn, Gly-Asp, Gly-Asp-Asp-Asp-Asp-Lys, Gly-Glu, Gly-Gly and their derivatives such as methyl, ethyl or benzyl esters or amides, Gly-Gly-Ala, Gly-Gly-Arg, Gly-Gly-Gly, Gly-Gly-Gly-Gly, Gly-Gly-Gly-Gly-Gly, Gly-Gly-Gly-Gly-Gly-Gly, Gly-Gly-Ile, Gly-Gly-Leu, Gly-Gly-Phe, Gly-Gly-Phe-Leu, Gly-Gly-Phe-Leu amide, Gly-Gly-Phe-Met, Gly-Gly-Phe-Met amide, Gly-Gly-sarcosine, Gly-Gly-Tyr-Arg, Gly-Gly-Val, Gly-His, Gly-His-Arg-Pro, Gly-His-Gly, Gly-His-Lys, Gly-His-Lys-OH, Gly-Ile, Gly-Leu amide, Gly-Leu, Gly-Leu-Ala, Gly-Leu-Phe, Gly-Leu-Tyr, Gly-Lys, Gly-Met, Gly-norLeu, Gly-norVal, Gly-Phe amide, Gly-Phe, Gly-Phe-Ala, Gly-Phe-Arg, Gly-Phe-Leu, Gly-Phe-Phe, Gly-Pro, Gly-Pro-Ala, Gly-Pro-Arg, Gly-Pro-Arg-Pro, Gly-Pro-Arg-Pro-OH, Gly-Pro-Gly-Gly, Gly-Pro-hydroxy-Pro, Gly-sarcosine, Gly-Ser, Gly-Ser-Phe, Gly-Thr, Gly-Trp, Gly-Tyr amide, Gly-Tyr, Gly-Tyr-Ala, Gly-Val, Gly-Phe-Ser, granuliberine R, growth hormone releasing factor and its fragments, Hexa-Ala, Hexa-Gly, Hippuryl-Arg (Hip-Arg), Hippuryl-Gly-Gly (Hip-

Gly-Gly), Hippuryl-His-Leu (Hip-His-Leu), Hippuryl-Lys, Hippuryl-Phe, hirudine and its fragments, His-Ala, His-Gly, His-Leu, His-Leu-Gly-Leu-Ala-Arg, His-Lys, His-Phe, His-Ser, His-Tyr, HIV envelope protein (gp120), Hydra peptides, P-hydroxyhippuryl-His-Leu, hypercalcemia malignancy factor (1-40), insulin chains B and C, P-iodo-Phe, Ile-Asn, Ile-Pro-Ile, insulin-like growth factor I (especially fragment 1-70), insulin-like growth factor II (especially its fragment 33-40), interleukin-1B fragment 163-171, isotocine, kassinine (Asp-Val-Pro-Lys-Ser-Asp-AGly-n-Phe-Val-Gly-Leu-Met-NH₂) katalcalcine (calcitonine precursor peptide), Tyr-katalcalcine, kemptide, kentsine, kyotorphine, laminine nonapeptide, laminine pentapeptide, laminine pentapeptide amide, leucine encephaline and related peptides, leucopyrokinine, Leu-Ala, Leu-beta-Ala, Leu-Arg, Leu-Asn, leucokinin I (Asp-Pro-Ala-Phe-Asn-Ser-Trp-Gly-NH₂) and II, Leucine-encephaline amide (Leu-encephaline amide) and related peptides, Leu-Gly, Leu-Gly-Gly, Leu-Gly-Phe, Leu-Leu amide, Leu-Leu, Leu-Leu-Leu amide, Leu-Leu-Leu, Leu-Leu-Phe amide, Leu-Leu-Tyr, Leu-Lys-Lys-Phe-Asn-Ala-Arg-Arg-Lys-Leu-Lys-Gly-Ala-Ile-Leu-Thr-Thr-Met-Leu-Ala, Leu-Met, Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys, Leu-Phe, Leu-Pro, Leu-Pro-Pro-Ser-Arg, Leu-Ser, Leu-Ser-Phe, Leu-Trp, Leu-Tyr, Leu-Val, leucotriene, Leu-Leu methyl ester, leupeptin, Leu-Ser-p-nitro-Phe-Nle-Ala-Leu methyl ester, beta-lipotropin fragments, litorine, luteinizing hormone releasing hormone and related peptides, lymphocyte activating pentapeptide, Lys-Ala, Lys-Ala 7-amido-4-methylcoumarin, Lys-Asp, Lys-Cys-Thr-Cys-Cys-Ala, Lys-Glu-Glu-Ala-Glu, Lys-Gly, Lys-Leu, Lys-Lys, Lys-Met, Lys-Phe, Lys-Pro-Pro-Thr-Pro-Pro-Glu-Pro-Glu-Thr, Lys-Serum thymic factor, Lys-Trp-Lys, Lys-Tyr-Trp-Trp-Phe amide, Lys-Val, macrophage inhibitory peptide (Tuftsin)

2067754

- 82 -

fragment 1-3, Thr-Lys-Pro), magainine I and II, mast cell degranulating peptide, mastoparan, alpha-mating factor, Melanocyte-Concentrating Hormone, MCD peptide, alpha-, beta-, gamma-, and delta-melanocyte stimulating hormones and related peptides, melittine, mesotocine, Met-beta-Ala, Met-Asn-Tyr-Leu-Ala-Phe-Pro-Arg-Met amide, methionine-enkephalin and related peptides, Met-Ala, Met-Ala-Ser, Met-Asn, methionine-enkephalin (Met-enkephalin, Tyr-Gly-Gly-Phe-Met) and related peptides, methionine-enkephalin amide (Met-Enkephalin amide, Tyr-Gly-Gly-Phe-Met-NH₂) and related peptides, Met-Gln-Trp-Asn-Ser-Thr-Thr-Phe-His-Gln-Thr-Leu-Gln-Asp-Pro-Arg-Val-Arg-Gly-Leu-Tyr-Phe-Pro-Ala-Gly-Gly, Met-Glu, Met-Gly, Met-Leu, Met-Leu-Phe, Met-Lys, Met-Met, Metorphamide, Met-Phe, Met-Pro, Met-Ser, Met-Tyr-Phe amide, Met-Val, N-Methoxycarbonyl-Nle-Gly-Arg, P-nitroaniline, methoxysuccinyl-Ala-Ala-Pro-Val, methoxysuccinyl-Ala-Ala-Pro-Val 7-amido-4-methylcoumarin, Met-somatotropine, molluscan cardioexcitatory peptide, morphiceptine, (Val³)-morphiceptine, motiline, MSH-release inhibiting factor, myeline basic protein or its fragments, naphthylamide-derivatives of various peptides, beta-naphthyl-Ala-Cys-Tyr-Trp-Lys-Val-Cys-Thr amide, alpha-neoendorphine, beta-neoendorphine, alpha-neurokinin, neurokinin A, (substance K, neuromedin L) and B, neoendorphine (alpha: Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro, beta, etc.) neuromedin B, C, K, U8, U-25 etc., neurokinin A and B, neuropeptides K and Y, neurophysin I and II, neurotensine and related peptides, nitroanilide peptide derivatives, Nle-Sta-Ala-Sta, NorLeu-Arg-Phe amide, opioid peptides (e.g. adrenal peptide E, Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-amides, casein fragments, casomorphine, N-CBZ-Pro-D-Leu, dermorphine, kyotorphine, morphiceptine (Tyr-Pro-Phe-Pro-NH₂), morphamide (Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val, adrenorphine),

osteocalcin (esp. its fragment 7-19), oxytocine and related peptides, pancreastatine and its fragments, such as 33-49, pancreatic polypeptide, pancreozymin, parathyroid hormone or fragments thereof, especially 1-34 and 1-84, penta-Ala, penta-Gly, penta-Phe, pepstatin A, peptide YY, peptide T, phalloidin, Phe-Ala-Ala-p-nitro-Phe-Phe-Val-Leu 4-pyridylmethyl ester, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe amide, Phe-Ala, Phe-Gly, Phe-Gly-Gly, Phe-Gly-Gly-Phe, Phe-Gly-Phe-Gly, Phe-Leu amide, Phe-Leu, Phe-Leu-Arg-Phe amide, Phe-Leu-Glu-Glu-Ile, Phe-Leu-Glu-Glu-Leu, Phe-Leu-Glu-Glu-Val, Phe-Met, Phe-Met-Arg-Phe amide, Phe-Phe, Phe-Phe-Phe, Phe-Phe-Phe-Phe, Phe-Phe-Phe-Phe-Phe, Phe-Pro, Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg, Phe-Tyr, Phe-Val, PHI-27, PHM-27, phosphoramidone, physalaemine (pGlu-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH₂), preproencephaline fragment 128-140, pressinoic acid and related peptides, Pro-Asn, proctoline (Arg-Tyr-Leu-Pro-Thr), proencephaline, Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys, Pro-Ala, Pro-Arg 4-methoxy-beta-naphthylamide, Pro-Asp, proglumide, Pro-Gly, Pro-Gly-Gly, Pro-hydroxy-Pro, Pro-Ile, Pro-Leu, Pro-Leu-Gly amide, Pro-Met, Pro-Phe amide, Pro-Phe, Pro-Phe-Arg 7-amido-4-methylcoumarin, Pro-Phe-Gly-Lys, Pro-Trp, Pro-Tyr, Pro-Val, cyclic AMP dependent protein kinase and its inhibitors, PyroGlu-Ala-Glu, PyroGlu-Ala, PyroGlu-Ala-Glu, PyroGlu-Asn-Gly, PyroGlu-Gly-Arg p-nitroanilide, PyroGlu-His-Gly amide, PyroGlu-His-Gly, PyroGlu-His-Pro amide, PyroGlu-His-Pro, PyroGlu-Lys-Trp-Ala-Pro, ranatensine, renine substrate tetradecapeptide, N-(alpha-rhamnopyranosyloxy-hydroxyphosphinyl) Leu-Trp, sarcosyl-Pro-Arg p-nitroanilide, sauvagine, sleep-inducing peptide (Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu), secretine and related peptides, Ser-Ile-Gly-Ser-Leu-Ala-Lys, Ser-Ser-Ser, serum thymic factor, Ser-Ala, Ser-beta-Ala, Ser-Asn, Ser-Asp, Ser-Asp-Gly-Arg-Gly, Ser-Glu, Ser-Gln, Ser-Gly,

Ser-His, Ser-Leu, Ser-Met, Ser-Phe, Ser-Ser-Ser, Ser-Tyr, sleep inducing peptide, somastotine and related peptides (e.g. cyclo(p-Trp-Lys-Trh-Phe-Pro-Phe), steroidogenesis activator polypeptide, substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂) and related peptides, N-succinyl-derivatives of various peptides, syndyphalin-20 (Tyr-D-Met(O)-Gly-Phe-ol), tentoxin, tetra-Ala, tetra-Gly, thiostrepton, DL-thiorphane (encephalinase inhibitor), Thr-beta-Ala, Thr-Asp, Thr-Leu, Thr-Lys-Pro-Arg, Thr-Ser, Thr-Ser-Lys, Thr-Tyr-Ser, Thr-Val-Leu, thymopoietin fragments, thymosin alpha and its fragments, thymus circulating factor, thyrocalcitonin, thyrotropin releasing hormone, tocinoic acid, tosylated peptides, transforming growth factors, Tri-Ala, Tri-Ala methyl ester, Trp-Ala, Trp-Ala-Trp-Phe amide, Trp-Glu, Trp-Gly, Trp-Gly-Gly, Trp-His-Trp-Leu-Gln-Leu, Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr, Trp-His-Trp-Leu-Ser-Phe-Ser-Lys-Gly-Glu-Pro-Met-Tyr, Trp-Leu, Trp-Met-Asp-Phe amide, Trp-norLeu-Arg-Phe amide, Trp-Phe, Trp-Trp, Trp-Tyr, Tuftsin (Thr-Lys-Pro-Arg) and its fragments, Tyr-Ala, Tyr-Ala-Gly, Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu, Tyr-Ala-Gly-N-methyl-Phe 2-hydroxyethylamide, Tyr-Ala-Phe-Met amide, Tyr-Arg, Tyr-atrioepetin II, Tyr-Glu, Tyr-Gly, Tyr-Gly-Ala-Val-Val-Asn-Asp-Leu, Tyr-Gly-Gly, Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Arg, Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val amide, Tyr-Gly-Trp-Phe-Phe amide, Tyr-Leu, Tyr-Phe, Tyr-Phe-Met-Arg-Phe amide, Tyr-Phe-Phe amide, Tyr-Pro-Leu-Gly amide, Tyr-Pro-Phe-Pro amide, Tyr-Pro-Val-Pro amide, Tyr-Thr-Gly-Leu-Phe-Thr, Tyr-Tyr-Phe amide, Tyr-Trp-Ala-Trp-Phe amide, Tyr-Trp-Ala-Trp-Phe methylamide, Tyr-Tyr-Leu, Tyr-Tyr-Phe, Tyr-Tyr-Tyr, Tyr-Tyr-Tyr methyl ester, Tyr-Tyr-Tyr-Tyr-Tyr-Tyr, Tyr-Val amide, Tyr-Val, Tyr-Val-Gly, Urodilatin, Urotensin II, Valosin, Val-Ala, Val-Ala p-nitroanilide, Val-Ala-Ala-Phe, Val-Asp, Val-Glu, Val-Gln, Val-Glu-Glu-Ala-Glu, Val-Glu-Ser-Ser-Lys,

Val-Gly, Val-Gly-Asp-Gln, Val-Gly-Gly, Val-Gly-Ser-Glu, Val-Gly-Val-Ala-Pro-Gly, Val-His-Leu-Thr-Pro, Val-His-Leu-Thr-Pro-Val-Glu-Lys, Val-Leu, Val-Lys, Val-Met, Val-Phe, Val-Pro, Val-Pro-Asp-Pro-Arg, Val-Pro-Leu, Val-Ser, Val-Thr, Val-Trp, Val-Tyr, Val-Tyr-Val, Val-Val, vasoactive intestinal peptides and related peptides, vasopressin related peptides, vasotocin and related peptides, xenopsin, etc.

Extended polypeptides are normally called proteins, independent of their detailed conformation. In this description, this term denotes, by and large, an enzyme or a coenzyme, an adhesion- or a recognition molecule, such as a CAMP or an OMP or a lectin, a histocompatibility complex, such as MHC-I or MHC-II, or an immunoglobuline (antibody) - or any (bio)chemical or (molecular)genetic modification thereof. Particularly useful for the applications according to this invention are the (bio)chemical modifications in which individual proteins are substituted with apolar residues, such as an alkyl, acyl, alkenoyl, etc. chains; but this is not a stringent limitation.

An enzyme is a catalytically active protein. Enzymes are normally grouped according to their basic functions. The most important enzymes for this invention are (E.C. numbers are given in brackets):

Oxidoreductases, such as: alcohol dehydrogenase (1.1.1.1), alcohol dehydrogenase (NADP dependent) (1.1.1.2), glycerol dehydrogenase (1.1.1.6), glycerophosphate dehydrogenase (1.1.1.8), xylulose reductase (1.1.1.10), polyol dehydrogenase (1.1.1.14), sorbitol dehydrogenase (1.1.1.14), myo-inositol dehydrogenase (1.1.1.18), uridine 5'-diphosphoglucose dehydrogenase

(1.1.1.22), glyoxalate reductase (1.1.1.26), lactate dehydrogenase (1.1.1.27), lactate dehydrogenase (1.1.1.28), glycerate dehydrogenase (1.1.1.29), beta-hydroxybutyrate dehydrogenase (1.1.1.30), beta-hydroxyacyl CoA dehydrogenase (1.1.1.35), malate dehydrogenase (1.1.1.37), malate enzyme (1.1.1.40), isocitric dehydrogenase (1.1.1.42), 6-phosphogluconate dehydrogenase (1.1.1.44), glucose dehydrogenase (1.1.1.47), beta-galactose dehydrogenase (1.1.1.48), glucose-6-phosphate dehydrogenase (1.1.1.49), 3alpha-hydroxysteroid dehydrogenase (1.1.1.50), 3beta-hydroxysteroid dehydrogenase (1.1.1.51), 3alpha,2beta-hydroxysteroid dehydrogenase (1.1.1.53), 3-phosphoglycerate dehydrogenase (1.1.1.95), fucose dehydrogenase (1.1.1.122), lactate dehydrogenase (cytochrome) (1.1.2.3), glucose oxidase (1.1.3.4), cholesterol oxidase (1.1.3.6), galactose oxidase (1.1.3.9), alcohol oxidase (1.1.3.13), glycolate oxidase (1.1.3.15), choline oxidase (1.1.3.17), glycerol-3-phosphate oxidase (1.1.3.21), xanthine oxidase (1.1.3.22), alcohol dehydrogenase (1.1.99.8), fructose dehydrogenase (1.1.99.11), formaldehyde dehydrogenase (1.2.1.1), formate dehydrogenase (1.2.1.2), aldehyde dehydrogenase (1.2.1.5), glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12), gabase (1.2.1.16), pyruvate oxidase (1.2.3.3), oxalate oxidase (1.2.3.4), dihydroorotate dehydrogenase (1.3.3.1), lipoxidase (1.3.11.12), alanine dehydrogenase (1.4.1.1), glutamic dehydrogenase (1.4.1.3), glutamate dehydrogenase (NADP) (1.4.1.4), L-amino acid oxidase (1.4.3.2), D-amino acid oxidase (1.4.3.3), monoaminoxidase (1.4.3.4), diaminoxidase (1.4.3.6), dihydrofolate reductase (1.5.1.3), 5,10-methylenetetrahydrofolat dehydrogenase (1.5.1.5), saccharopine dehydrogenase NAD+ (1.5.1.7), octopine dehydrogenase (1.5.1.11), sarcosine oxidase (1.5.3.1),

sarcosine dehydrogenase (1.5.99.1), glutathione reductase (1.6.4.2), ferridoxin-NADP+ reductase (1.6.7.1), NADPH-FMN oxidoreductase (1.6.99.1), cytochrome c reductase (1.6.99.3), NADH-fmn oxidoreductase (1.6.99.3), dihydropteridin reductase (1.6.99.7), uricase (1.7.3.3), diaphorase (1.8.1.4), lipoamide dehydrogenase (1.8.1.4), cytochrome oxidase (1.9.3.1), nitrate reductase (1.9.6.1), phenolase (1.10.3.1), ceruloplasmine (1.10.3.2), ascorbate oxidase (1.10.3.3), NADH peroxidase (1.11.1.1), catalase (1.11.1.6), lactoperoxidase (1.11.1.7), myeloperoxidase (1.11.1.7), peroxidase (1.11.1.7), glutathione peroxidase (1.11.1.9), chloroperoxidase (1.11.1.10), lipoxidase (1.13.1.12), protocatechuate 3,4-dioxygenase (1.13.11.3), luciferase (glow-worm) (1.13.12.7), salicylate hydroxylase (1.14.13.7), p-hydroxybenzoate hydroxylase (1.14.13.2), luciferase (bacterial) (1.14.14.3), phenylalanine hydroxylase (1.14.16.1), dopamine-beta-hydroxylase (1.14.17.1), tyrosinase (1.14.18.1), superoxide dismutase (1.15.1.1), ferredoxine-NADP reductase (1.18.1.2), etc.. Transferases, such as: catecholic o-methyltransferase (2.1.1.6), phenylethanol-amine N-methyl-transferase (2.1.1.28), aspartate transcarbamylase (2.1.3.2), ornithine carbamyltransferase (2.1.3.3), transketolase (2.2.1.1), transaldolase (2.2.1.2), choline acetyltransferase (2.3.1.6), carnitine acetyltransferase (2.3.1.7), phosphotransacetylase (2.3.1.8), chloroamphenicol acetyltransferase (2.3.1.28), kanamycine 6'-acetyltransferase (2.3.1.55), gentamicine acetyltransferase (2.3.1.60), transglutaminase (2.3.2.13), gamma-glutamyl transpeptidase (2.3.2.2), phosphorylase A (2.4.1.1), phosphorylase B (2.4.1.1), dextransucrase (2.4.1.5), sucrose phosphorase (2.4.1.7), glycogen synthase (2.4.1.11), uridine 6'-diphosphoglucuronyltransferase (2.4.1.17), galactosyl trans-

ferase (2.4.1.22), nucleoside phosphorylase (2.4.2.1), orotidine-5'-monophosphate pyrophosphorylase (2.4.2.10), glutathione S-transferase (2.5.1.18), glutamine-oxalate transaminase (2.6.1.1), glutamic-pyruvate transaminase (2.6.1.2), gabase (2.6.1.19), hexokinase (2.7.1.1), galactokinase (2.7.1.6), fructose-9-phosphate kinase (2.7.1.11), gluconate kinase (2.7.1.12), phosphoribulokinase (2.7.1.19), NAD kinase (nicotinamide adenine dinucleotide kinase) (2.7.1.23), glycerokinase (2.7.1.30), choline kinase (2.7.1.32), protein kinase (3':5'-cyclic-AMP dependent) (2.7.1.37), phosphorylase kinase (2.7.1.38), pyruvate kinase (2.7.1.40), fructose-9-phosphate kinase (pyrophosphate dependent) (2.7.1.50), acetate kinase (2.7.2.1), carbamate kinase (2.7.2.2), 3-phosphoglyceric phosphokinase (2.7.2.3), creatine phosphokinase (2.7.3.2), etc.

Transpeptidases, such as: esterase (3.1.1.1), lipase (3.1.1.3), phospholipase A (3.1.1.4), acetyl esterase (3.1.1.6), cholinesterase, acetyl (3.1.1.7), choline-esterase, butyryl (3.1.1.8), pectinesterase (3.1.1.11), cholesterol esterase (3.1.1.13), glyoxalase ii (3.1.2.6), phosphatase, alkaline (3.1.3.1), phosphatase acid (3.1.3.2), 5'-nucleotidase (3.1.3.5), 3'-nucleotidase (3.1.3.6), glucose-6-phosphatase (3.1.3.9), fructose-1,6-diphosphatase (3.1.3.11), phytase (3.1.3.26), phosphodiesterase i (3.1.4.1), glycerophosphorylcholine (3.1.4.2), phospholipase C (3.1.4.3), phospholipase D (3.1.4.4), deoxyribonuclease I (3.1.4.5), deoxyribonuclease II (3.1.4.6), ribonuclease N1 (3.1.4.8), sphingomyelinase (3.1.4.12), phosphodiesterase 3':5'-cyclic (3.1.4.17), phosphodiesterase II (3.1.4.18), endonuclease (3.1.4.21), ribonuclease A (3.1.4.22), ribonuclease B (3.1.4.22), 3'-phosphodiesterase 2':3'-cyclic nucleotide (3.1.4.37), sulfatase (3.1.6.1), chondro-4-sulfatase (3.1.6.9),

chondro-6-sulfatase (3.1.6.10), ribonuclease T2
 (3.1.27.1), ribonuclease T1 (3.1.27.3), ribonuclease u2
 (3.1.27.4), nuclease (3.1.30.1), nuclease, (from
 micrococces) (3.1.31.1), alpha-amylase (3.2.1.1), beta-
 amylase (3.2.1.2), amyloglucosidase (3.2.1.3), cellulase
 (3.2.1.4), laminarinase (3.2.1.6), dextranase (3.2.1.11),
 chitinase (3.2.1.14), pectinase (3.2.1.15), lysozyme
 (3.2.1.17), neuraminidase (3.2.1.18), alpha-glucosidase,
 maltase (3.2.1.20), beta-glucosidase (3.2.1.21), alpha-
 galactosidase (3.2.1.22), beta-galactosidase (3.2.1.23),
 alpha-mannosidase (3.2.1.24), beta-mannosidase
 (3.2.1.25), invertase (3.2.1.26), trehalase (3.2.1.28),
 beta-N-acetylglucosaminidase (3.2.1.30), beta-glucuronidase
 (3.2.1.31), hyaluronidase (3.2.1.35), beta-
 xylosidase (3.2.1.37), hesperidinase (3.2.1.40),
 pullulanase (3.2.1.41), alpha-fucosidase (3.2.1.51),
 mycodextranase (3.2.1.61), agarase (3.2.1.81), endoglyco-
 sidase F (3.2.1.96), endo-alpha-N-acetylgalactosamini-
 dase (3.2.1.97), NADase (nicotinamide adenine glycopepti-
 dase) F (3.2.2.5), dinucleotidase (3.2.2.18), thiogluc
 (3.2.3.1), s-adenosylhomocystein-hydrolase (3.3.1.1),
 leucin-aminopeptidase, (from cytosol) (3.4.11.1), leucin-
 aminopeptidase, microsomal (3.4.11.2), pyroglutamate-
 aminopeptidase (3.4.11.8), carboxypeptidase a (3.4.12.2),
 carboxypeptidase B (3.4.12.3), prolidase (3.4.13.9),
 cathepsin C (3.4.14.1), carboxypeptidase W (3.4.16.1),
 carboxypeptidase A (3.4.17.1), carboxypeptidase B
 (3.4.17.2), alpha-chymotrypsin (3.4.21.1), beta-
 chymotrypsin (3.4.21.1), gamma-chymotrypsin (3.4.21.1),
 delta-chymotrypsin (3.4.21.1), trypsin (3.4.21.4),
 thrombin (3.4.21.5), plasmin (3.4.21.7), kallikrein
 (3.4.21.8), enterokinase (3.4.21.9), elastase from
 pancreas (3.4.21.11), protease (subtilisin) (3.4.21.14),
 urokinase (3.4.21.31), elastase from leucocytes
 (3.4.21.37), cathepsin B, (3.4.22.1), papain (3.4.22.2),

ficin (3.4.22.3), bromo-elain (3.4.22.4), chymopapain (3.4.22.6), clostripain (3.4.22.8), proteinase A (3.4.22.9), pepsine (3.4.23.1), renine (3.4.23.4), cathepsin D (3.4.23.5), protease (aspergillopeptidase) (3.4.23.6), collagenase (3.4.24.3), collagenase (3.4.24.8), pinguinain (3.4.99.18), renine (3.4.99.19), urokinase (3.4.99.26), asparaginase (3.5.1.1), glutaminase (3.5.1.2), urease (3.5.1.5), acylase I (3.5.1.14), cholyglycine hydrolase (3.5.1.24), urease(ATP-hydrolyzing) (3.5.1.45), penicillinase (3.5.2.6), cephalosporinase (3.5.2.8), creatininase (3.5.2.10), arginase (3.5.3.1), creatinase (3.5.3.3), guanase (3.5.4.3), adenosine-deaminase (3.5.4.4), 5'-adenylate acid-deaminase (3.5.4.6), creatinine deiminase (3.5.4.21), anorganic pyrophosphatase (3.6.1.1), adenosine 5'-triphosphatase (3.6.1.3), apyrase (3.6.1.5), pyrophosphatase, nucleotide (3.6.1.9), etc.

Lyases, such as: pyruvate-decarboxylase (4.1.1.1), oxalate decarboxylase (4.1.1.2), oxalacetate decarboxylase (4.1.1.3), glutamic decarboxylase (4.1.1.15), ornithine decarboxylase (4.1.1.17), lysine decarboxylase (4.1.1.18), arginin decarboxylase (4.1.1.19), histidine decarboxylase (4.1.1.22), orotidine 5'-monophosphate decarboxylase (4.1.1.23), tyrosine decarboxylase (4.1.1.25), phospho(enol) pyruvate carboxylase (4.1.1.31), ribulose-1,5-diphosphate carboxylase (4.1.1.39), phenylalanine decarboxylase (4.1.1.53), hydroxymandelonitrilelyase (4.1.2.11), aldolase (4.1.2.13), N-acetylneuramine acid aldolase (4.1.3.3), etc. citrate lyase (4.1.3.6), citrate synthase (4.1.3.7), tryptophanase (4.1.99.1), isozymes of carbonic anhydrase (4.2.1.1), fumarase (4.2.1.2), aconitase (4.2.1.3), enolase (4.2.1.11), crotonase (4.2.1.17), delta-amino-levulinate dehydratase (4.2.1.24), chondroitinase ABC

(4.2.2.4), chondroitinase AC (4.2.2.5), pectolyase (4.2.2.10), aspartase (4.3.1.1), histidase (4.3.1.3), phenylalanine ammonia-lyase (4.3.1.5), argininosuccinate lyase (4.3.2.1), adenylosuccinate lyase (4.3.2.2), glyoxalase II (4.4.1.5), isomerases, such as: ribulose-5'-phosphate 3-epimerase (5.1.3.1), uridine 5'-diphosphogalactose 4-epimerase (5.1.3.2), mutarotase (5.1.3.3), triosephosphate isomerase (5.3.1.1), phosphoriboisomerase (5.3.1.6), phosphomannose isomerase (5.3.1.8), phosphoglucose isomerase (5.3.1.9), tautomerase (5.3.2.1), phosphoglucomutase (5.4.2.2), ligases, e.g.: aminoacyl-tRNA synthetase (6.1.1), s-acetyl coenzyme A synthetase (6.2.1.1), succinic thiokinase (6.2.1.4), glutamine synthetase (6.3.1.2), pyruvate carboxylase (6.4.1.1), etc.

The following are, amongst others, referred to as proteases: aminopeptidase M, amino acid-arylamidase, bromo-elaine, carboxypeptidase A, carboxypeptidase B, carboxypeptidase P, carboxypeptidase Y, cathepsine C, chymotrypsine, collagenases, collagenase/dispace, dispace, elastase, endoproteinase Arg-c, endoproteinase Asp-n sequencing grade, endoproteinase Glu-c (proteinase V8), endoproteinase Glu-c sequencing grade, endoproteinase Lys-c, endoproteinase Lys-c sequencing grade, endoproteinases, factor Xa, ficine, kallikrein, leucine-aminopeptidase, papaine, pepsine, plasmin, pronase, proteinase K, proteinase V8 (endoproteinase Glu-c), pyroglutamate-aminopeptidase, pyroglutamate-aminopeptidase, restriction protease factor Xa, subtilisine, thermolysine, thrombine, trypsin, etc.

A coenzyme according to this invention is any substance which supports enzyme activity. Amongst the biologically important coenzymes are, for example, acetyl-coenzyme A,

acetylpyridine-adenine-dinucleotide, coenzyme A, flavine-adenine-dinucleotide, flavine-mononucleotide, NAD, NADH, NADP, NADPh, nicotinamide-mononucleotide, s-palmitoyl-coenzyme A, pyridoxal-5'-phosphoric acid, etc.

Another class of proteins, which are important in the context of this invention, are lectins. Plants, and sometimes also animal, tissues are suitable sources of lectins; particularly convenient sources are *Abrus precatorius*, *Agaricus bisporus*, *Agrostemma githago*, *Anguilla anguilla*, *Arachis hypogaea*, *Artocarpus integrifolia*, *Bandeiraea simplicifolia* BS-I und BS-II, (*Griffonia simplicifolia*), *Banhlula purpurea*, *Caragana arborescens*, *Cicer arietinum*, *Canavalia ensiformis* (jack bean), *Caragana arborescens* (Siberian pea tree), *Codium fragile* (green algae), *Concanavalin A* (Con A), *Cytisus scoparius*, *Datura stramonium*, *Dolichos biflorus*, *Erythrina corallodendron*, *Euonymus europaeus*, *Gelonium multiflorum*, *Glycine max* (soy), *Griffonia simplicifolia*, *Helix aspersa* (garden snail), *Helix pomatia* (escargot), *Laburnum alpinum*, *Lathyrus odoratus*, *Lens culinaris* (lentil), *Limulus polyphemus*, *Lycopersicon esculentum* (tomato), *Lotus tetragonolobus*, *Luffa aegyptiaca*, *Maclura pomifera* (Osage orange), *Momordica charantia* (bitter pear melon), *Naja mocambique* (Mozambiquan cobra), *Naja Naja kaouthia*, *Mycoplasma gallisepticum*, *Persea americana* (avocado), *Phaseolus coccineus* (beans), *Phaseolus limensis*, *Phaseolus lunatus*, *Phaseolus vulgaris*, *Phytolacca americana*, *Pseudomonas aeruginosa* PA-I, *Pisum sativum* (pea), *Ptilota plumosa* (red algae), *Psophocarpus tetragonolobus* (winged bean), *Ricinus communis* (castor bean), *Robinia pseudoacacia* (false acacia, black locust), *Sambucus nigra* (clematis), *Saponaria officinalis*, *Solanum tuberosum* (potato), *Sophora japonica*, *Tetragonolobus purpureus* (winged or asparagus pea), (*Lotus tetragono-*

lobus), *Triticum vulgare* (wheat germ), *Ulex europaeus*, *Vicia faba*, *Vicia sativa*, *Vicia villosa*, *Vigna radiata*, *Viscum album* (mistle), *Wisteria floribunda*, etc.

Further interesting proteins are, e.g. the activator of tissue-plasminogen, insulin, kallikrein, keratin, kininogen, lactoterrin, laminarin, laminin, alpha₂-macroglobulin, alpha₁-microglobulin, F₂-microglobulin, high density lipoproteins, basic myelin-protein, myoglobins, neurofilaments I, II, and III, neurotensin, oxytocin, pancreatic oncofoetal antigen, parvalbumin, plasminogen, platelet factor 4, pokeweed antiviral protein, porphobilinogen, prealbumin, prostate specific antigens, protamine sulfate, protein C, protein C activator, protein S, prothrombin, retinol binding protein, S-100 protein, pregnancy protein-1, serum amyloid A, serum amyloid P component, tenascin, testosterone-estradiol binding globulin, thioredoxin, thrombin, thrombocytin, beta-thromboglobulin, thromboplastin, microsomal antigen from thyroid, thyroid stimulating hormone, thyroxine binding globulin, transcortin, transferrin, ubiquitin, vimentin, vinculin, vitronectin, etc.

Some typical examples of human and animal hormones which can be used as agents according to the invention are, for example, acetylcholine, adrenaline, adrenocorticotrophic hormone, angiotensin, antidiuretic hormone, cholecystokinin, chorionic gonadotropin, corticotropin A, danazol, diethylstilbestrol, diethylstilbestrol glucuronide, 13,14-dihydro-15-keto-prostaglandins, 1-(3',4'-dihydroxyphenyl)-2-aminoethanol, 5,6-dihydroxytryptamine, epinephrine, follicle stimulating hormone, gastrin, gonadotropin, β -hypophamine, insulin, juvenile hormone, 6-ketoprostaglandins, 15-ketoprostaglandins,

LTH, luteinizing hormone releasing hormone, luteotropic hormone, α -melanocyte stimulating hormone, gamma-melanocyte stimulating hormone, 5-melanocyte stimulating hormone, noradrenaline, norepinephrine, oxytocine, parathyroid hormone, parathyroid substances, prolactine, prostaglandins, secretine, somatostatine, somatotropine (STH), thymosine alpha 1, thyrocalcitonine, thyroglobuline, thyroid stimulating hormone, thyrotropic hormone, thyrotropine releasing hormone, 3,3',5-triiodothyroacetic acid, 3,3',5'-triiodothyronine, TSH, vasopressine, etc.

Oestrogens are mostly steroid hormones with 18 carbon atoms and one unsaturated (aromatic) ring. Amongst the most important oestrogens are, for example, chlorotrianisene, diencestrole, diethylstilboestrole, diethylstilboestrol-dipropionate, diethylstilboestrol-disulfate, dimestrole, estradiol, estradiolbenzoate, estradiolundecylate, estriolsuccinate, estrone, ethinglestradiol, nexoestrole, nestranole, oestradiolvalerate, oestriole and quineestrole.

Gestagenes are typically synthetic hormones, mainly with progesterone-like characteristics; the most important agents belonging to this class are allylestrenole, chloromadinonacetate, dimethisterone, ethisterone, hydroxyprogesteron-caproate, lynestrenole, medrogestone, medroxyprogesteron-acetate, megestrolacetate, methyllestrenolone, norethisterone, norethisterone-acetate, and norgestrel.

Agents can also be parts of a biological extract. As sources of biologically and/or pharmacologically active extracts, the following are worth-mentioning: for example, *Acetobacter pasteurianum*, *Acokanthera ouabaio*

2069754

cathel, Aesculus hippocastanum, Ammi visnaga Lam., Ampel
Huasca, Apocynum Cannabium, Arthrobotrys superba var.
oligospora (ATCC 11572), Atropa belladonna, Bacillus
Lentus, Bacillus polymyxa, Bacillus sphaericus, Castilleja
elastica cerv., Chondrodendron tomentosum (Ampel Huasca),
Convallaria majalis, Coronilla-enzymes, Corynebacterium
hoagii (ATCC 7005), Corynebacterium simplex, Curvularia
lunata (Wakker) Boedijn, Cylindrocarpon radicola (ATCC
11011), Cynara scolymus, Datura Metel, didymella,
digitalanidase, digitalis Lanata, digitalis purpurea,
Duboisia, Flavobacterium dehydrogenans, Fusarium
exquisetii saccardo, Hyoscyamus niger, Jaborandi-leaves
(P. microphyllus Stapf), Micromonospora purpurea u.
echinospora, Paecilomyces varioti Bainier var.
antibioticus, Penicillium chrysogenum Thom,
Penicillium notatum Westling, Penicillium patulum,
Rauwolfia serpentina Benth., Rhizopus arrhizus Fischer
(ATCC-11145), Saccharomyces cerevisiae, Schizomycetes
ATCC-7063, Scilla maritima L., Scillarenase, Septomyxa
affinis (ATCC 6737), Silybum marianum Gaertn., Strepto-
myces ambofaciens, Strophantusgratus, Strophantus Kombe,
Thevetia peruviana, Vinca minor L., Vinca rosea, etc.

Unless stated otherwise, all substances, surfactants, lipids,
agents or additives with one or several chiral carbon atoms
can be used either as a racemic mixture or in the form of
optically pure enantiomers.

WORKING PRINCIPLE

The transport of agents through permeation barriers can be
mediated by such carriers which fulfill the following basic
criteria:

- carriers should experience or create a gradient which drives them into or through a barrier, e.g. from the body surface into or through the skin, or from the surface of a leaf into the depth of a leaf, or from one side of a barrier to the other;
- the resistance to permeation which is felt by the carriers in the barrier should be as small as possible in comparison to the driving force;
- carriers should be capable of permeating in and/or through a barrier without thereby losing their associated agents in an uncontrollable manner.

Carriers, moreover, should preferably provide control of the distribution of agents, as well as over the effectiveness and temporal development of the agents action. They should be capable of bringing materials into the depth of and across a barrier, if so desired, and/or should be capable of catalyzing such a transport. Last but not least, such carriers should affect the range and depth of action as well as the type of cells, tissue parts, organs and or system parts which can be reached or treated, under suitable conditions at least.

In the first respect, chemical gradients are especially convenient for biological applications. Particularly suitable are the physico-chemical gradients, such as the pressure of (de)hydration pressure (humidity gradient) or a difference in concentration between the sites of application and action; however, electrical or magnetic fields as well as thermal gradients are also interesting in this respect. In technological applications, an externally applied pressure or existing hydrostatic pressure difference are also of importance.

In order to fulfill the second condition, carriers must be sufficiently 'fluid' at the microscopic scale; this enables them to easily cross the constrictions in the permeability barrier.

Permeation resistance is a decreasing function of the decreasing carrier size. But also the carrier driving force frequently depends on the size of the permeating particle, droplet or vesicle; when the driving pressure is size-independent, the corresponding force also typically decreases with decreasing carrier size. This causes the transfer effectiveness to be a complex function of the carrier size, often showing a maximum depending on the chosen carrier and/or agent composition.

In the case of molecular aggregates the permeation resistance is largely determined by the mechanical elasticity and deformability of the carrier, the viscosity of the total preparation being also important, however. The former must be sufficiently high, the latter low enough.

Size and, even better, deformability can serve as a criterion for the optimization of the supramolecular carriers according to this invention. As an indication of deformability, the capacity of individual carriers to form protrusions can be studied, as a function of all relevant system parameters. (In practical terms, it is often sufficient to investigate only such variables which come into question for a controllable application. The examples given in this application, therefore, only pertain to varying the concentrations of the edge active components and the absolute carrier concentration which affect the forced diminishment of the lipid vesicle or of vesicle permeation.) This is true e.g. for transcutaneous and transcuticular transport as well as for the transport of agents through the lung alveoli, into the hair, into gels, and

the like.

With regard to the third requirement, the choice of the carriers, agents and additives, as well as the applied carrier dose or concentration all play some role. Low dose, in the majority of cases, gives rise to a predominantly surface treatment: poorly water-soluble substances in such case remain confined largely to the apolar region of a permeability barrier (such as in the epidermal membranes); agents which are highly soluble and can diffuse easily from the carriers can attain a distribution which is different from that of the carrier particles; for such substances, the permeability of a transfersomal membrane is also important. Edge active substances with a tendency to leave carriers and move into a barrier give rise to a locally variable carrier composition, etc. These interdependencies should be thought of and considered prior to each individual application. In the search for a set of conditions under which a simple carrier vesicle becomes a transfersome, the following rules of thumb can be used:

- At first, the conditions are determined under which the carrier vesicles are solubilized by the edge active substances. At this critical point the 'vesicles' are maximally deformable owing to the fact that they are permanently formed and deformed. At the same time, however, they are also unstable and incapable of holding and transferring water soluble substances.
- Next, the carrier composition or concentration is adapted by reducing the edge activity in the system to an extent which ensures the vesicle stability as well vesicle deformability to be sufficiently high; this also ensures the permeation capacity of such carriers to be satisfactory. The term stability in this application implies,

on the one hand, a mechanical tendency of the carrier components to "stay together"; on the other hand, that the carrier composition during the transport, and in particular during the permeation process, does not change at all or not much. The position of the corresponding optimum which one is looking for hereby depends on many boundary conditions. The type of agent molecules also plays an important role in this. The smaller and the more hydrophilic the agent to be transported, the further the carrier system must be spaced from the solubilization point; the desired shelf life of carriers is also important: upon approaching the solubilization point, the tendency of transfersomes to form larger particles may increase and the carrier's storage capacity simultaneously decrease.

- Ultimately, the system parameters need to be optimized with respect to the envisaged modes and goals of a given application. Rapid action requires a high permeation capability; in order to achieve slow drug release, it is advantageous to ensure gradual penetration through the permeability-barrier and a correspondingly 'finely adjusted' membrane permeability; in order to reach deep regions, high doses are needed; in order to obtain a broad distribution, it is recommended to use carrier concentrations which are not too high.

This application describes some relevant properties of the transfersomes as carriers for the lipid vesicles. Most of the examples pertain to carriers made of phospholipids, but the general validity of conclusions is not restricted to this carrier or molecule class. The vesicle examples should only illustrate the requirements which should be fulfilled in order to attain penetration through permeability barriers, such as skin. Similar properties, moreover, ensure carrier transport

across animal or human epidermis, mucosa, plant cuticle, inorganic membranes, etc.

The fact that the cells in a horny skin layer continuously merge with the watery compartments of subcutis is probably one reason for the spontaneous permeation of transfersomes through the 'pores' in this layer: during the permeation process transfersomes are propelled by the osmotic pressure. As an alternative, external pressures, such as an electroosmotic or hydrostatic pressure, however, can also be applied in addition.

Depending on the vesicle dose used, the dermally applied carrier particles can penetrate as deep as the subcutaneous layer. Agents can then be locally released, enriched in (the depth of) the application site, or forwarded to other tissues and body systems through a system of blood and lymph vessels, the precise drug fate being dependant on the carrier size, composition and formulation.

It is sometimes convenient to adjust the pH-value of a formulation immediately after it has been prepared or directly prior to an application. Such an adjustment should prevent the deterioration of individual system components and/or drug carriers under the conditions of initial pH; simultaneously, a physiological compatibility should be achieved. For the neutralization of carrier suspensions, physiologically tolerable acids or bases are most frequently used as well as buffers with a pH-value between 3-12, preferably 5 to 9 and most often 6-8, depending on the goal and site of application. Physiologically acceptable acids are, for example, diluted aqueous solutions of mineral acids, such as hydrochloric acid, sulfuric acid, or phosphoric acid, or organic acids, such as carboxyalkane acids, e.g. acetic acid. Physiologically acceptable bases are, for example, diluted sodium hydroxide,

suitably ionized phosphoric acids, etc.

Formulation temperature is normally chosen to be well suited for the given substances; for aqueous preparations it is normally in the range of 0 to 95°C. Whenever possible, one should work in the temperature range 18-70°C; particularly preferred are temperatures between 15 and 55°C for the work with fluid chain lipids; the preferred temperature range for the lipids with ordered chains is from 45 to 60°C. Other temperature ranges are possible, however, most notably for the non-aqueous systems or preparations containing cryo- or heat-stabilizers.

If required by the sensitivity of one of the system components, transfersome formulations can be stored in cold (e.g. at 4°C). It is, moreover, possible to make and keep them under an inert atmosphere, e.g. under nitrogen. Shelf-life, furthermore, can be extended if no substances with multiple bonds are used, and if the formulation is (freeze) dried, or if a kit of dry starting materials is dissolved or suspended and processed at the site of application only.

In the majority of cases, carriers are applied at room temperature. But applications at lower or higher temperatures are also possible, especially when synthetic substances are used.

Transfersomal preparations can be processed previously or at the site of application, as has been described, for example, in our previous German patent application P 40 26 833.0-43, and exemplified in several cases in the handbook on 'Liposomes' (Gregoriadis, G., Edits. CRC Press, Boca Raton, Fl., Vols 1-3, 1987), in the monography 'Liposomes as drug carriers' (Gregoriadis, G., Edits. John Wiley & Sons, New York, 1988), or in the laboratory manual 'Liposomes. A Practical Approach' (New, R., Oxford-Press, 1989). If

required any suspension of drugs, moreover, can be diluted or concentrated (e.g. by per ultracentrifugation or ultrafiltration) immediately prior to a final application; additives can also be given into a preparation at this or a previous time. Upon any such manipulation, however, a possible shift of the permeation optimum for a given carrier preparation must be taken into account or prevented.

Transfersomes as described in this applications are well suited to be used as carriers of lipophilic substances, such as fat-soluble biological agents, therapeutics, poisons, etc. But it is quite likely that transfersomes used in combination with water soluble substances, especially when the molecular weight of the latter exceeds 1000 Dt, will be of even greater practical value.

Transfersomes, moreover, can contribute to the stabilization of substances which are sensitive to hydrolysis; they can improve carrier and drug distribution in the specimen and at the site of application and can also ensure a more favourable effect of the drug in time. Basic carrier ingredients can also bring advantages of their own. However, the most important carrier characteristics is the capability of transporting materials into and through a permeability barrier; this opens up a way for applications which prior to this discovery were not feasible.

The specific formulations as described in this invention have been optimized for the topical use on - or in the vicinity of - (a) permeability barrier(s). Particularly interesting barriers of this kind are skin and plant cuticle. (But formulations according to this invention are also well suited for the peroral (p.o) or parenteral (i.v. i.m. or i.p.) application, especially when edge active substances have been chosen in order to keep the drug loss at the site of

application low.) Edge active substances which have a diminished activity, are degraded preferentially, are absorbed particularly efficiently or are diluted strongly at the site of application are especially valuable in this last respect.

In dermatology, application doses of up to 50, often up to 10 and very frequently less than 2.5 (or even less than 1 mg) of carrier substance are used per cm^2 of skin surface, the given masses pertaining to the basic carrier substance. The optimal mass depends on the carrier composition, desired penetration depth and duration of action, as well as on the detailed application site. Application doses useful in agrotechnics are typically lower and frequently below 0.1g pro m^2 .

Depending on the goal of application, each formulation can also contain suitable solvents up to a total concentration which is determined by certain plausible physical (no solubilization or appreciable shift of penetration optimum), chemical (no lowering of stability), or biological and physiological (little adversary side effects) formulation requirements.

Quite suitable for this purpose are, for example, the unsubstituted or substituted, e.g. halogenated, aliphatic, cycloaliphatic, aromatic or aromatic-aliphatic hydrocarbons, such as benzol, toluol, methylene chloride or chloroform, alcohols, such as methanol or ethanol, propanediol, erithritol, short-chain alkane carboxylic acid esters, such as acetic acid acid alkylesters, such as diethylether, dioxan or tetrahydrofuran, or mixtures thereof.

A survey of the lipids and phospholipids which can be used for the applications as described in this report in addition to the ones already mentioned is given, for example, in 'Form and

function of phospholipids' (Ansell & Hawthorne & Dawson, eds.), 'An Introduction to the Chemistry and Biochemistry of Fatty Acids and Their Glycerides' of Gunstone and in other reference books. All implicitly and explicitly mentioned lipids and surfactants as well as other suitable edge active substances and their preparation are well known. A survey of available surfactants, together with the trademarks under which they are marketed by their manufacturers, is given in the annals 'Mc Cutcheon's, Emulsifiers & Detergents', Manufacturing Confectioner Publishing Co. An up-to-date compilation of the pharmaceutically acceptable agents is given, for example, in 'Deutsches Arzneibuch' (and in the annually updated list 'Rote Liste'); furthermore, in the British Pharmaceutical Codex, European Pharmacopoeia, Farmacopoeia Ufficiale della Repubblica Italiana, Japanese Pharmacopoeia, Nederlandse Pharmacopoeia, Pharmacopoeia Helvetica, Pharmacopée Française, The United States Pharmacopoeia, The United States NF, etc. A concise list of suitable enzymes can be found in the volume on 'Enzymes', 3rd Edition (M. Dixon and E.C. Webb, Academic Press, San Diego, 1979); more recent developments are described in the series 'Methods in Enzymology'. Many examples of the glycohydate-binding proteins which could be interesting for the use in combination with carriers as described in this invention are quoted in 'The Lectins: Properties, Functions, and Applications in Biology and Medicine' (I.E. Liener, N. Sharon, I.T. Goldstein, Eds. Academic Press, Orlando, 1986) as well as in the corresponding special publications; substances which are particularly interesting for agrotechnical applications are described, for example, in 'The Pesticide Manual' (C.R. Worthing, S.B. Walker, Eds. British Crop Protection Council, Worcestershire, Englande, 1986, e.g. 8th edition) and in 'Wirkstoffe in Pflanzenschutz und Schädlingsbekämpfung', which is published by Industrie-Verband Agrar (Frankfurt); most commonly available antibodies are listed in the catalogue

'Linscott's Directory', the most important neuropeptides in 'Brain Peptides' (D.T. Krieger, M.J. Brownstein, J.B. Martin, Eds. John Wiley, New York, 1983), corresponding supplementary volumes (e.g. 1987) and other special journals.

Methods for the preparation of liposomes, which in the majority of cases can also be used for manufacturing transfersomes, are described, for example, in 'Liposome Technology' (Gregoriadis, Ed., CRC Press) or older books dealing with similar topics, such as 'Liposomes in Immunobiology' (Tom & Six, Eds., Elsevier), 'Liposomes in Biological Systems' (Gregoriadis & Allison, Eds., Willey), 'Targeting of Drugs' (Gregoriadis & Senior & Trouet, Plenum), etc. Corresponding patent publications also are a valuable source of relevant information.

The following examples are aimed at illustrating this invention without restricting it. All temperatures are in degrees Celsius, carrier sizes in nanometers, pressures in Pascal and other units in standard SI system.

Ratios and percentages are given in moles, unless otherwise stated.

Examples 1-13:

Composition:

250-372 mg	phosphatidylcholine from soy-bean (+95 % = PC)
187-34.9 mg	oleic acid (+99 %)
0.312-0.465 ml	ethanol, absolute
10 mM	Hepes

Preparation:

Increasing amounts of oleic acid were pipetted into different volumes of alcoholic PC-solutions containing 75 micromoles of lipid so as to create a concentration series with a lipid/surfactant ratio beginning with $L/S=0.5$ and increasing by 0.2 units in each step. Subsequently, each lipid sample was supplemented with 4.5 ml of sterile buffer solution and the mixtures were incubated at 4°C for one day. When the pH value had to be adjusted by addition of 1 M NaOH, the first incubation period was followed by another incubation for 24 hours. In order to obtain a final liposome suspension, each sample was thoroughly mixed and filtered through a polycarbonate filter (0.45 micrometer) into a glass vial which was then kept closed at 4°C.

Characterization:

Permeation resistance is assumed to be proportional to the relative pressure needed to perform a secondary filtration through a 0.2 micrometer filter. In this report this resistance is given in relative units of 1 to 10.

Vesicle size is measured by means of dynamic light scattering at 33 degrees C, using a Malvern Zeta-Sizer instrument. For the analysis of correlation curves, a special variant of the software package "Contin" is employed.

In this experimental series all vesicle sizes are relatively independent of the total concentration of edge active substances, in the range of 300 through 350 nm.

Permeation:

Permeation resistance first increases with decreasing relative concentration of fatty acid in the transfersomes. This trend is not monotonous, however. At a lipid/surfactant-ratio of

2067754

- 107 -

approx. 2, the liposome permeation capacity starts to increase; but it then decreases again until, for L/S above 3, the transfersomes have nearly lost their capability for passing through narrow constrictions. Vesicles with a lipid/surfactant molar ratio of 1/2 are nearly perfectly permeable, however. (A suspension with 8 % lipid in such case can be filtered nearly as easily as pure water.). At this concentration ratio, which corresponds roughly to 30 % of the solubilization dose of fatty acids in an alkaline suspension, liposomes thus appear to correspond to optimal transfersomes.

Specific data points (0) are shown in figure 1. Vesicles diameters were always measured after permeation experiments.

Examples 14-20:

Composition:

349-358 mg	phosphatidylcholine from soy-bean (+95 % = PC)
63.6-52.2 mg	oleic acid (+99 %)
10 mM	Hepes

Preparation:

4.5 ml of buffer in each case are pipetted to a corresponding amount of lipids and fatty acids to create a concentration series with L/S = 1.92 through 2.4 in the steps of 0.08 units each; the pH value is set to 7.2-7.3 by 1 M NaOH. Lipid suspension after an incubation for 6 days at 4°C is treated by ultrasonication until vesicles with an average diameter of 0.8 micrometers are formed.

Permeation and Characterization:

Permeation resistance is determined as described in examples

2067754

- 108 -

1-13. Its value, as a function of the concentration of edge active substance in the system resembles the results of measurements 1-13. The resulting vesicles are somewhat larger than in the previous set of experiments, however, having diameters in the order of 500 nm. This can be explained by the relatively slow material flow during filtration.

Corresponding measured points are shown as (+) in figure 1.

Examples 21-31:

Composition:

322.6-372 mg	phosphatidycholine from soy-bean (+95 %=PC)
96.8-34.9 mg	oleic acid (+99 %)
0.403-0.465 ml	ethanol, absolute
10 mM	Hepes
130 mM	NaCl, p.a.

Preparation:

Preparation procedure used essentially corresponds to the one of examples 14-20. The main difference is that the electrolyte concentration in the present case was isotonic with blood.

Permeation and Characterization:

The measured permeation resistance corresponds, within the limits of experimental error, to the results given in examples 1-13. Vesicle sizes are also similar in both cases. Immediately after the lipid vesicle have been formulated, their diameters are in the range of 320-340 nm. 8 days later, however, the vesicle size has increased to approx. 440 nm.

2067754

- 109 -

Corresponding experimental data is given in figure 2.

Examples 32-39:

Composition:

184.5-199.8 mg	phosphatidylcholine from soy-bean (+95% PC)
20.5-22.2 mg	phosphatidylglycerol from egg PC (puriss., Na-salt, =PG)
44.9-26.1 μ l	oleic acid (+99 %)
0.165-0.178 ml	ethanol, absolute
4.5 ml	Hepes, 10 mM

Preparation:

Anhydrous PG is mixed with an alcoholic solution of PC to give a clear solution with 90 % PC and 10 % PG. Oleic acid is added to this solution; the resulting lipid/surfactant ratios are between 1.6 and 2.8; an isomolar specimen is made in addition to this. All mixtures are suspended in 4.5 ml of a sterile buffer solution to yield a final lipid concentration of 4 % and then left for 3 days, after a pH-value adjustment with NaOH, in order to age.

Permeation and Carrier Characteristics:

For determining the permeation resistance, the same procedure as in examples 1-13 is used. All measured values are, as a rule, smaller than in the case of carriers which contained no charged species but had a similar L/S-ratio. Based on our experiments with a 4 % suspension of PC and oleic acid we conclude that the relatively low total lipid concentration plays only a minor role in this respect.

As in previous examples, a resistance minimum is observed for

the 4 % PC/PG mixtures; this minimum, however, is found with L/S-ratios which are by some 20 % higher than those measured with 8 % lipid suspensions. Vesicle diameters, however, hardly differ from those measured in examples 1-13.

Precise permeation data is shown in figure 3. All quoted diameters were measured immediately after individual permeation experiments. But even 40 days later, they are hardly bigger than at the beginning; figure 4 illustrates this.

Examples 40-49:

Composition:

301.3-335.4 mg	phosphatidylcholine from soy-bean (+95%PC)
123.3-80.8 µl	Tween 80 (puriss.)
0.38-0.42 ml	ethanol, absolute
4.5 ml	phosphate buffer, isotonic, sterile

Preparation:

Increasing volumes of Tween 80 are pipetted into appropriate volumes of an alcoholic PC solution. This gives rise to a concentration series with 12.5 through 25 mol-% surfactant (L/S = 4-8). In addition to this, samples with L/S=2 and 3 are also made. After the addition of buffer, lipid vesicles are formed spontaneously: prior to further use, these are made somewhat smaller, with the aid of a 0.8 micrometer filter.

Permeation and Carrier Characteristics:

Permeation resistance is determined in the previously described manner. The corresponding values (0) are shown in the left part of figure 5. As in the case of transfersomes

which contain oleic acid, a region of anomalously high permeation capability (at $L/S = 6$) can be seen relatively far away from the solubilization point. But it is not before below $L/S=4$ that a maximum permeability is observed. The transfersomal optimum thus is located in a range which differs by a factor of 1.5-2 from the solubilization point.

Precise permeation data is given in figure 5 (wide lines, left panel). The experimental data in right panel documents the vesicle diameters determined after permeability measurements.

Examples 50-61:

Composition:

314.2-335.4 mg	soy-bean phosphatidylcholine (+95 % = PC)
107.2-80.8 μ l	Tween 80 (puriss.)
4.5 ml	phosphate buffer, isotonic, sterile

Preparation:

First Tween 80 and subsequently phosphate buffer are added to appropriate quantities of PC. The resulting mixture is agitated at room temperature for 4 days. The further procedure is as described in examples 40-49.

Permeation and Carrier Characteristics:

Corresponding permeability data is given in figure 5 (thin lines). It confirms, by and large, the results of experiments nos. 40-49.

Examples 62-75:

Composition:

- 112 -

Preparation:

Vesicle permeation through constrictions and vesicle solubilization:

Corresponding measured data is shown in figure 6. It indicates that the permeation resistance of transfersomes with an L/S ratio below 3.5/1 is very small but that this resistance increases significantly at higher L/S values (left panel); the increase of the mean vesicle diameter above L/S = 2.75 (right panel) is probably a consequence of the decreased flow (and thus of a diminished hydrodynamic shear) caused by the greater permeability resistance in this concentration range.

Within only a few hours after preparation the size of vesicles

just above the solubilization limit (at L/S between 1.25/1 and 2.5/1) is significantly bigger than in the vicinity the 'transfersome optimum'. Such undesired consequences of surfactant activity (cf. Fromherz, P. in: 'Galstone disease, Pathophysiology and Therapeutic Approaches', pp. 27-33, Springer, Berlin, 1990) should always be taken into account. At L/S of approx. 1.25/1, solubilization sets in which leads to the formation of, in our case unmeasurably, small mixed micelles of a size of approximately 5 nm.

Examples 76-91:

Composition:

1.627-0.5442 g phosphatidylcholine from soy-bean (grade I, S100)
4.373-0.468 g Na-cholate, puriss.
60 ml phosphate buffer (physiological)

Preparation:

A 10 % suspension of S100 in phosphate buffer is ultrasonicated at room temperature until the mean vesicle size is approx. 350 nm.

This suspension is divided into three equal volume parts containing 10 %, 1 % and 0.2 % phospholipids. Starting with these preparations, aliquots containing 5 ml of suspension each are prepared. These are supplemented with increasing amounts of sodium cholate (partly in the form of a concentrated micelle suspension), yielding a concentration series with L/S ratios between 1/5 and 5/1. Prior to each permeation- and solubilization measurement, the starting suspension is aged for 1 week at 4°C.

Vesicle permeation through constrictions and vesicle

solubilization:

In order to determine the permeation resistance of these samples two different procedures are used.

In the first series, each suspension is diluted prior to an actual measurement to get a final lipid concentration of 0.2 %; subsequently it is pressed through a filter with a pore size of 0.1 micrometers. The sample resistance is identified with the inverse value of the volume which has passed through the filter pores during a period of 5 minutes.

In the second series, the permeation resistance is determined as in examples 1-13 and finally renormalized by dividing the values thus obtained with regard to the final lipid concentration.

The resulting data shows that both the solubilization limit and the position of a 'transfersome optimum' expressed in terms of preferred L/S ratios are dependent on the overall lipid concentration. In the case of a 10 % suspension the corresponding values are approx. 1/1 and 2.75/1, respectively; for the 0.2 % suspension they increase to 1/4 and 1/1, however.

Examples 92-98:Composition:

16.3-5.4 mg phosphatidylcholine from soy-bean (Grade I,
S100)
41.5-5.5 mg Na-desoxycholate, puriss.
5 ml phosphate buffer (physiological)

Preparation:

A suspension of 1 % desoxycholate containing vesicles is prepared as described in examples 76-91.

Vesicle permeation through constrictions and vesicle solubilization:

The measurements of this experimental series show that vesicles containing desoxycholate are solubilized already at L/S ratios near 1/2, i.e. at an L/S ratio which is by a factor of 2-3 lower than in the case of S100/Na-cholate vesicles.

Examples 99-107:

Composition:

3 mM Suspension of phosphatidylcholine from soy-bean
(grade I, S100) in phosphate buffer Na-cholate, puriss.

Preparation:

A 3 mM suspension of S100 in phosphate buffer is partly homogenized at room temperature. 3 ml of this suspension are supplemented each with increasing amounts of sodium cholate in order to create a series with increasing L/S ratios between 1/2 and 12/1. After three days of incubation, these aliquots are ultrasonicated at 55°C, using a 50 % duty-cycle; simultaneously, the optical density at 400 nm of each sample is recorded. An analysis of the resulting experimental data within the framework of a bimodal exponential model reveals two characteristic vesicularization rates (τ_1 and τ_2); these characterize the temporal dependence of the number of lamellae in each vesicle (τ_1) and the changes in the mean size of vesicles (τ_2).

Vesicle characterization and deformability.

The tau 1 and tau 2 values represented in figure 7 show that the mechanical properties of transfersomes, which are reflected in the value of parameter tau 2, exhibit a similar L/S dependence as the solubilization and permeation tendency (cf. fig. 6). For a 0.2 % suspension investigated in this series 1 cholate molecule per lipid is required for a rapid formation of vesicles (for the formation of largely unilamellar vesicles).

Examples 108-119:Composition:

121.2-418.3 mg	phosphatidylcholine from soy-bean (Grade I, PC)
378.8-81.7 mg	Triton X-100
4.5 ml	0.9 % NaCl solution in water

Preparation:

A 10 % PC-suspension in isotonic solution of sodium chloride is homogenized at 22°C until the mean size of lipid vesicles is approx. 400 nm. This suspension is then distributed in aliquots of approx. 4.8 ml. A sufficient volume of Triton X-100 is pipetted into each of these aliquots to give a concentration series with nominal PC/Triton ratios in the range of 0.25 through 4 in steps of 0.5. All resulting samples are occasionally mixed and incubated at 4°C for 14 days.

Vesicle solubilization

The optical density (OD (400 nm)) of a lipid-triton mixture after a 10-fold dilution provides insight into the vesicle

solubilization; this is represented in the right panel of figure 8. The solubilization limit is approx. 2 triton molecules per PC-molecule. Right below this limit, the optical density (OD (400 nm)) - and thus the vesicle diameters - attain the greatest values. At PC/triton ratios higher than 2,5/1, the change in the optical density of given suspensions is only minimal.

Vesicle permeation and characteristics:

In order to evaluate the permeation capability of the resulting lipid vesicles and transfersomes all suspensions were pressed through fine-pore filters (0.22 micrometer), as described in examples 1-13. The required pressure increases gradually with the decreasing total triton concentration in the suspension; for L/S ratios higher than 2/1 this significantly limits the permeation capability of carriers.

Corresponding results are summarized in the left half of figure 8.

Examples 120-128:

Composition:

403,5-463,1 mg	dipalmitoyl tartaric acid ester, Na-salt
96,5-36,9 mg	laurylsulfate, Na-salt (SDS)
4,5 ml	triethanolamine buffer, pH 7.5

Preparation:

In this test series a synthetic lipid, which is not found in biological systems, was chosen to be the basic transfersome constituent. For each experiment the required dry lipid mass was weighed into a glass vial and mixed with 4.5 ml of buffer.

The latter contained sufficient amounts of sodiumdodecylsulfate (SDS) to give various L/S ratios between 2/1 and 6/1. Well mixed suspensions were first kept at room temperature for 24 hours and subsequently mixed again thoroughly.

Permeation capacity and vesicle characteristics:

Liposomes were pressed through a 0.2 micrometer filter. Simultaneously, the permeation resistance was measured. Vesicles with an L/S ratio below 4/1 can pass the membrane pores very easily; in contrast to this, all vesicles with lower surfactant concentrations or vesicles without edge active components can pass through the porous constrictions only with difficulty (not before an excess pressure of 5 MPa has been created) or not at all (membranes burst).

Examples 129-136:

Composition:

101,6-227 mg	phosphatidylcholine from soy-bean
148,4-22,2 mg	octyl-glucopyranoside (β -octylglucoside),
puriss. 9,85 ml	phosphate buffer, pH 7,3
	ethanol, absolute

Preparation:

Phosphatidylcholine in ethanol (50 %) and octyl-glucopyranoside were mixed in different relative ratios in order to prepare a concentration series with increasing L/S values between 1/4 and 2/1 (and a final total lipid concentration of 2.5 %). Each lipid mixture in a glass vial was then supplemented with 4.5 ml of buffer. Subsequently, the resulting suspension was mixed in an agitator for 48 hours

at 25°C. The suspension turbidity was greater for the specimen containing lower amounts of octylglucoside. A fine sediment formed in standing samples. Each suspension was mixed thoroughly before the experiment.

Vesicle permeation and characteristics:

All suspensions can be filtered without any problem through filters with a pore diameter of 0.22 micrometer, using only minimal excess pressures of less than 0.1-0.2 MPa; the only two exceptions are the samples with the lowest surfactant concentration. These give rise to small permeation resistances which on the renormalized scale (cf. figures 1-5) corresponds to values of approx. 1 and 2.5, respectively. Figure 9 presents said data.

If the pore diameter is reduced to 0.05 micrometers only suspensions with L/S ratios below 2/1 can still be filtered.

Irrespective of the pore size used all preparations with L/S ratios below 2/1 are unstable; after only a few days, a phase separation is observed between a micelle rich and a vesicle rich phase.

Examples 137-138:

Composition:

43,3 mg, 50 mg	phosphatidylcholine from soy-bean
0.5 mg	phosphatidylethanolamine-N-fluorescein
6,7 mg, 0 mg	cholate, Na-salt, p.a.
5 ml	Hepes-buffer, pH 7,3

Preparation:

Phosphatidylcholine with the addition of 1 %-fluoresceinated lipids with or without desoxycholate is suspended in 5 ml buffer. The lipid/surfactant ratio is 3.5/1 or 1/0. Both 1 %-suspensions are then ultrasonicated in a glass vial for 1.5 or 15 minutes (25 W, 20°C), until the mean vesicle size is approx. 100 nm.

Spontaneous vesicle permeation:

Onto a Millipore-filter with 0.3 micrometer pore diameter, mounted into a Swinney-holder, the lower half of which has been wetted and filled with water, 50 microliters of a lipid suspension are pipetted through the upper opening. By a gentle swinging motion, a relatively homogeneous sample distribution on the filter surface is ensured. After 30 minutes, the holder is carefully opened and left to dry for 60 minutes. Subsequently the water from below the filter is collected and checked fluorimetrically (excitation 490 nm, emission 590 nm). (The determined light intensity is a measure of the permeation capacity.)

The transport of fluorescence markers mediated by surfactants containing transfersomes gives rise to a fluorescence signal of 89.5; in control experiment a value of 44.1 is established. This indicates that transfersomes are capable of transporting encapsulated substances across permeability barriers.

Examples 137-139:

Composition:

43,5, 45,3, 50 mg	phosphatidylcholine from soy-bean
0.5 mg	phosphatidylethanolamine-N-fluorescein
6,5, 4,7, 0 mg	desoxycholate, Na-salt, p.a.

25 ml Hepes-buffer, pH 7,3

Preparation and results:

Lipid vesicles are made and tested as described in examples 137-138. Measurements show that the transfersomes which contain deoxycholate already show similarly good results at a characteristic L/S ratio of 5/1 as transfersomes which contain cholate at a ratio of L/S=3.5.

Examples 140-142:

Composition:

50 mg; 43,3 mg; 15,9 mg	phosphatidylcholine from soy-bean
0.5 mg	phosphatidylethanolamine-N-fluorescein
0 mg; 6,7 mg; 34,1 mg	cholate, Na-salt, p.a.
5 ml	Hepes-buffer, pH 7,3

Preparation:

Lipid vesicles consisting of phosphatidylcholine and a fluorescent additive were made as in examples 137-138. For this experiment, suspensions with a lipid/surfactant ratio of 1/0, 4/1 and 1/4 were used. The former two contained fluorescent lipid vesicles, the latter a micellar suspension.

Spontaneous penetration into plant leaves:

A fresh onion is carefully opened in order to gain access to individual leaves; these correspond to low-chlorophyll plant leaves. For each measurement, 25 microliters of a fluorescinated suspension are applied onto the concave (inner or upper) side of each onion leaf; as a result of this a

convex droplet with an area of approx. 0.25 square centimeters is formed. (Carriers which contain surfactants can be easily identified owing to their higher wetting capability.) After 90 minutes the (macroscopically) dry lipid film is eliminated with the aid of a water stream from a jet-bottle with a volume of 50 ml.

After this treatment, the 'leaf surface' attains a slightly reddish appearance in the case of surfactant containing transfersomes as well as mixed micelles. Leaves incubated with surfactant-free vesicles cannot be distinguished from the untreated leaves.

Fluorescence measurements using a red filter (excitation through a blue filter from above) show that leaves which were covered with transfersomes are intensively fluorescent throughout the treated area. In certain places extremely brilliant aggregates are detected; these probably correspond to the non-eliminated vesicle-clusters. The fluorescence of leaves which were treated with a surfactant solution in some places is comparably intensive; at other positions their fluorescence is weaker, however, than in the case of transfersome-treated leaves.

The leaves which were treated with standard lipid vesicles do not fluoresce. Over large surface areas they are indistinguishable from the non-treated leaf regions.

This shows that transfersomes can transfer lipophilic substances spontaneously and irreversibly into a plant leaf or its surface. Their penetration capacity exceeds that of preparations containing highly concentrated surfactants, i.e. well established 'membrane fluidizers'.

Examples 143-145:

Composition:

50 mg; 43,5mg; 17,1 mg phosphatidylcholine from soy-bean
0.5 mg phosphatidylethanolamine-N-fluorescein
0 mg; 4,7 mg; 32,9 mg desoxycholate, Na-salt, p.a.
5 ml Hepes-buffer, pH 7,3

Preparation and results:

The preparation and results are identical with those of experiments 140-142.

Examples 146-148:Composition:

50 mg; 36,4; 20 mg phosphatidylcholine from soy-bean
0.5 mg phosphatidylethanolamine-N-fluorescein
0 mg; 13,6 mg; 30 mg Brij 35
5 ml Water

Preparation and results:

Preparation and results are comparable to those of experiments 140-142 and 143-145.

Examples 146-150:Composition:

84,2 to 25 mg phosphatidylcholine from soy-bean 80 %
75 kBq Giberellin A4, 3H-labelled
15,8 to 75 mg polyoxyethylene (23)-laurylether (Brij 35)
1 ml water

ethanol, absolute

Preparation:

An ethanolic lipid solution (50 %) is mixed with a corresponding amount of an ethanolic solution of giberellin and suspended in 1 ml of water or in appropriate volumes of a surfactant suspension to obtain a total lipid concentration of 10 % and L/S ratios of 8/1, 4/1, 2/1, 1/1 and 1/2. The resulting (mixed) suspension is then briefly homogenized with the aid of ultrasound so that the mean vesicle size is always below 300 nm.

Carrier suspensions are distributed over the surface of 3 leaves of *Ficus Benjaminii*; there, they are permitted to dry for 6 hours. After subsequent intensive washing of each leaf surface with 5 ml of water per square centimetre and destaining with a peroxide solution, the radioactivity in the homogenized plant material is measured scintigraphically in a beta-counter.

Agent transport in plant leaves:

Experiments show, as in examples 140-142, that transfersomes can bring the agent molecules into a leaf surface much more effectively than a micellar solution.

Examples 151-157:

Composition:

32,8-0.64 mg	phosphatidylcholine from soy-bean (purity higher than 95 %, PC)
75 kBq	dipalmitoylphosphatidylcholine tritium- labelled

2,2-34,4 mg bile acid, Na-salt, p.a.
0.32 ml phosphate buffer, pH 7,3

Preparation:

In each case, 35 mg of lipid are mixed with tritium-labelled dipalmitoylphosphatidylcholine in chloroform. After thorough drying under vacuum, the resulting mixture is suspended in 0.32 ml of buffer; the nominal surfactant/lipid ratios are 0; 0.125; 0.167; 0.263; 0.5 and 1 mol/mol. All suspensions are ultrasonicated until they are comparably opalescent, with the exception of the last, optically clear micellar solution. (The time for efficient necessary sonication decreases with increasing S/L). Control measurements with non-radioactive suspensions indicate that the mean 'particle' size in all samples must be around 100 nm. In all experiments approximately 1 day old suspensions are used.

Penetration into and through the intact skin:

On the back of an immobilized nude-mouse anaesthetized with ether six areas of 1x1 cm are marked. Each of these areas is covered with 20 microliters of a carrier suspension at 3x5 minutes intervals. 60 minutes later, the mouse is killed. From each treated area a sample is excised which is then cut to pieces, solubilized and de-stained. The skin-associated radioactivity is measured scintigraphically.

The corresponding results are summarized in figure 10. For comparison, the normalized values are also given which were taken from our patent application pertaining to the use of liposomes for topical anaesthesia. Optimal transfersomes are appreciably better than non-optimal preparations containing surfactants.

Examples 158-162:Composition:

31 mg	phosphatidylcholine from soy-bean (purity higher than 95 %, PC)
75 kBq	dipalmitoylphosphatidylcholine tritium- labelled
4 mg	deoxycholate, Na-salt, p.a.
0.32 ml	phosphate buffer, pH 7,3

Preparation:

In each case 35 mg of lipid (PC and deoxycholate) are mixed with tritium-labelled dipalmitoylphosphatidylcholine in a chloroform solution. The resulting lipid mixture is dried and then dissolved in 30 microliters of warm, absolute ethanol. This solution is then mixed with 0.32 ml of a buffer solution (phosphate buffer, 10 mM, 0.9 % NaCl); this corresponds to a lipid/surfactant ratio of 4/1. The resulting suspension is thoroughly mixed and subsequently filtered through filters with pore sizes of 0.8; 0.45; 0.22 and 0.1 micrometers; this gives rise to vesicles with diameters of approx. 800, 400, 200 or 100 nm (suspensions A, B, C, D).

Penetration into and through the skin:

Tails of 2 anaesthetized mice are treated with 50 microlitres of a corresponding vesicle suspension for 15 minutes. Two control animals obtain an i.v. injection of 0.2 ml 1/10 diluted suspension B. After 30, 60, 120, 180, 240 and 360 minutes, blood specimens are drawn from the tail-tip. The radioactivity of these samples, which is determined by means of beta-scintigraphy, is a reliable indication of the systemic concentration of carrier-associated, radioactively labelled

lipids.

Experimental data show (fig. 11) that systemically applied transfersomes are eliminated from blood comparably as rapidly as standard liposomes. The size of carrier particles appears not to affect the spontaneous penetration into skin. All transfersomes investigated in this study can penetrate intact skin and get into the depth of a body quite effectively within a period of 4 hours at approx. 1 carrier; tendency increasing.

Examples 163-165:

Composition:

88 mg	phosphatidylcholine from soy-bean (purity higher than 95 %, PC)
75 kBq	inulin, tritium labelled
12 mg	deoxycholate, Na-salt, p.a.
100 ml	ethanol, absolute
0.9 ml	isotonic salt solution

Preparation:

100 mg of PC dissolved in 100 ml of warm ethanol, or a corresponding PC/deoxycholate solution (L/S = 4.5), are mixed with 0.9 ml of an isotonic salt solution (suspensions A and B, respectively). Each suspension is ultrasonicated until the mean vesicle size is about 150 nm.

12 microlitres of an aqueous solution of tritium-labelled inulin are pipetted into 38 microliters of a freshly prepared suspension of empty liposomes (A) or transfersomes (B). Subsequently, all mixtures are sonicated in closed vials for 60 minutes in an ultrasound bath at room temperature; they are all used for experiments within 24 after vesicle preparation.

Spontaneous inulin transfer through the skin:

On the abdomen of NMRI-mice in general anaesthesia, which three days before were depilated using medical tweezers, 10 microlitres of a vesicle suspension containing inulin in every case are applied twice at time intervals of approx. 3-5 minutes.

15, 30, 60, 120, 180, 240, 300 and 360 minutes later, 0.05 ml of blood are routinely taken from the tail of a each mouse to be then investigated scintigraphically. 6 hours later the subcutaneous tissues at the application site, as well as liver and spleen of all animals of this experiment are collected. After solubilization and decolouring procedures, these organs are also checked scintigraphically.

The results of this study are collected in figure 12. They show that normal liposomes can hardly mediate a percutaneous inulin uptake; in contrast to this, 6 hours later approx. 1.4 % of this marker which was applied in the form of transfersomes are found in the blood. This transfer sets in approximately 2-3 hours after the application and is not yet completed 6 hours after each application.

After 6 hours in the case of transfersomes, an average of 0.8 % (this corresponds to 24.1 % of the recovered dose) are in the skin at the application site; 0.9 % are found in the liver; spleen contains less than 0.1 % of the absolute dose. In the body (blood, spleen, liver) approximately 73.8 % of the recovered dose are thus found again.

In contrast to this, approximately 2 % of the normal liposomes at the application site can be detected by eye, the corresponding doses in the liver and spleen being below

0.1 %. This corresponds to a recovery of 95.3 % at the application site and 6.7 % of this dose in the body of the test animal.

Example 166:

Composition:

386 mg	phosphatidylcholine from soy-bean (purity > 95 %)
58.5 mg	sodium-cholate (L/S - 3,5)
500 µl	ethanol (96 %)
2.25 ml	0.9 % NaCl solution (per inject.)
2.25 ml	Actrapid HM 40 (corresponds to 90 I.U. of recombinant human insulin)

Preparation:

Samples are prepared essentially as described in examples 62-75. A mixture of aqueous salt solution and human recombinant insulin (with 6.75 mg m-cresole) is mixed with a lipid solution in ethanol. The resulting, opaque suspension is aged over night. 12 hours later, this suspension is pressed through a sterile filter (Anodisc, pore diameter 0.2 micrometers) with the aid of nitrogen gas with excess pressure of 0.25 MPa under sterile conditions to be then filled into the glass container.

The nominal lipid/surfactant ratio is 3.5; the calculated molar surfactant concentration in the lipid double layer is approx. 5/1. This corresponds to 50 % of the concentration required for solubilization.

The mean radius of vesicles in final suspension in this experiment was 97 nm.

Application:

0.5 ml of a fresh, insulin containing transfersome suspension are applied onto the untreated skin of the left forearm of an informed, healthy male volunteer aged 37 years (starved for 18 hours) and distributed over an area of approx. 10 cm². 5 minutes later, additional 300 microlitres of identical suspension are positioned in two halves on the forearm and upper arm, respectively. 5-10 minutes later, the suspension on the upper arm (dose approx. 2,5 mg/cm²) has almost completely disappeared; it has thus nearly completely penetrated into skin. In contrast to this, lipids applied onto the forearm (dose approx. 7.5 mg/cm²) are still well perceptible.

Activity:

In order to assess the biological activity of insulin, approx. 2 hours before the sample application, a permanent, soft catheter is placed into a vein in the right hand. Every 15-45 minutes, 1-1.5 ml of blood are collected from this catheter; the first 0.5-1 ml thereof are discarded; the remaining 0.5 ml are measured with a standard enzymatic glucose test. In each case three determinations with three to four independent specimens are made. The corresponding experimental data is summarized in figure 13. It shows that transfersomes mediate a significant hypoglycemia in the peripheral blood some 90 minutes after the drug application; this effect lasts for approx. 2 hours and amounts to approx. 50 % of the magnitude of the hypoglycemic effect of a comparable dose of subcutaneously applied insulin; the effect of the former lasts 200 % longer, however.

Examples 167-172:

Composition:

956 mg	phosphatidylcholine from soy-bean (+95 %)
0-26 mg	sodium-desoxycholate
1 mg	prostaglandine E1
1 ml	ethanol absolute
50 ml	0.9 % NaCl solution (per inject.)

Preparation:

1 ml of ethanol is pipetted into a glass flask with 1 mg of prostaglandine. After thorough mixing, the resulting prostaglandine solution is transferred to the appropriate amount of dry lipid in another glass vial. The original flask is flushed once again with the new lipid/prostaglandine solution and subsequently supplemented with 6 ml of an isotonic salt solution. The prostaglandine containing flask is washed twice with 2 ml of 0.9 % NaCl and mixed with the original lipid suspension. The sample is then divided into 5 parts; into individual aliquots sodium-desoxycholate is added at concentrations of 0; 1.6; 3.25; 6.5 or twice 13 mg/ml.

The resulting 10 % suspensions are aged for 24 hours. Subsequently they are either ultrasonicated or filtered manually through a 0.2 micrometer-filter, depending on cholate concentration. The specimens with the highest surfactant concentration are either filtered or ultrasonicated. Finally, the samples are diluted to obtain a final PGE1 concentration of 20 micrograms/ml and kept in dark glass bottles in a refrigerator. Vesicle radius right after sample preparation is 85 nm, two months later 100 nm.

Application and Action:

In each experiment 0.25 ml of a lipid suspension are applied on neighbouring but not interconnected regions of abdominal skin. 10 minutes later the skin surface is macroscopically dry; 15 minutes later, some of the application sites show a reddish appearance which, according to the test person's statement, is associated with a weak local pain. The intensity of oedema grades as 0, 0, 0, 0-1, 3 and 3 points (on a scale from 1-10).

This shows that merely transfersomes - but not liposomes or sub-optimal surfactant-containing vesicles - can penetrate into intact skin and thereby transfer drugs into body. The precise mode of sample preparation plays no role in this.

Examples 173-175:

Composition:

79.4 mg; 88.5 mg	phosphatidylcholine from soy-bean (+95%)
20.6 mg, 11.5 mg	sodium-deoxycholate
10 µg	hydrocortison
0.1 ml	ethanol absolute
1 ml	phosphate buffer, physiological

Preparation:

Lipids and hydrocortison are mixed as approx. 50 % ethanolic solution and subsequently supplemented with 0.95 ml of phosphate buffer. The resulting, very heterogeneous suspension is treated with ultrasound (25 W, 3-5 min). Specimens with an L/S ratio of 2/1 can be homogenized with ease, specimens with L/S = 4/1 are relatively difficult to homogenize.

Specimens with 1 and 2.5 weight-% result in stable suspensions

independent of the precise L/S ratio; 10 weight-% of agent cannot be incorporated into stable transfersomes of the given composition.

Examples 175-200:

Composition:

1.1 - 2mg phosphatidylcholine from soy-bean (+95%=PC)
0 - 32.5 mol-% Tween 80
pH 7.2 isotonic phosphate buffer

Preparation:

Different amounts of phospholipid and surfactant in each experiment are weighed or pipetted into 25 ml of buffer at ratios which yield suspensions with 0 - 32,5 mol-% of Tween 80 and a constant total lipid concentration of 2 %. Specimens are sterilized by filtering, filled into sterile glass vials and aged for 4 through 34 days. Then, the optical density of each sample is determined. This depends strongly on surfactant concentration but hardly on time within the framework of measuring conditions.

Characterization:

23 specimens each containing 3 ml of an individual lipid suspension are ultrasonicated in closed vials in a bath sonicator. Three, four and six hours later the samples' optical density is determined. Such measurements are repeated with every new sample series after the relative sample positions were exchanged in a systematic manner; the determination of optical density, again, is performed three, four and six hours after the start of sonication. All values corresponding to one concentration are summed up and divided

by the number of measurements; the resulting value is a measure of the samples' capacity for vesicularization under given conditions.

This procedure is an alternative or a supplement to the permeation resistance measurements as described in examples 40-49. Figure 16 shows, for example, that the amount of surfactant required for good mechanical deformability in the case of Tween 80 is 2-3 times lower than the corresponding solubilization concentration. This result is in good accord with the results of the permeation experiments.

Examples 201-215

Composition:

256.4-447 mg	phosphatidylcholine from soy-bean (+95% PC)
243.6-53.1 mg	Brij 96
0.26-0.45 ml	ethanol, absolute
4.5 ml	phosphate buffer, pH 6,5, 10 mM

Preparation:

Increasing volumes of Brij 96 are pipetted into the corresponding volumes of an alcoholic PC solution. Thus, a concentration series is obtained with L/S values between 1/1 and 1/8. After the addition of a buffer very heterogeneous liposomes are formed which are homogenized by means of filtering through a 0.2 μ m filter.

Permeation and carrier characteristics:

The already described method for the determination of suspensions permeability resistance is used. Corresponding values are given in the left panel of figure 14 as circles or

crosses (two independent test series). The functional dependence of the samples' permeability resistance as a function of the L/S ratio is similar to that of comparable transfersomes and is illustrated in the right panel of figure 14. The maximum permeation capacity is not reached before the L/S-value is below 3.

Examples 216-235

Composition:

202,0-413 mg	phosphatidylcholine from soy-bean (+95% = PC)
298,0-87,0 mg	Myrj 49
0.26-0.45 ml	ethanol, absolute
4.5 ml	phosphate buffer, pH 6,5, 10 mM

Preparation and Characterization:

Transfersomes are made and characterized as described for examples 201-215. Their permeation properties as a function of the relative surfactant concentration in the individual specimen is given in the left panel of figure 15. The right panel gives corresponding equilibrium values; the latter, however, provide no information about vesicle suitability for permeation and agent transport.

Example 236:

Composition:

144,9 mg	phosphatidylcholine from soy-bean
24.8 mg	desoxycholate, Na-salt
1.45 ml	Actrapid HM 100 (145 I.U.)
0.16 ml	ethanol, absolute

Preparation:

Appropriate quantities of both lipids are dissolved in corresponding amounts of ethanol and mixed with a standard solution of insulin. 12 hours later, the crude carrier suspension is homogenized by means of filtration. Average vesicle diameter is 225 ± 61 nm and nominal insulin concentration is 83 I.U. Over an area of appr. 10 square centimeters on the right forearm 0.36 ml (30 I.U.) of insulin in transfersomes are distributed. Blood samples are taken every 10 minutes through a heparinized soft catheter positioned in a vein in the right forearm; the first 0.5 ml are always discarded; the following 0.5-0.8 ml of each sample are sedimented and immediately frozen; the remainder of each sample is used for the determination of blood glucose concentration during the experiment.

Activity:

These liposomes with a relatively high surfactant concentration have only a very limited capability of transporting insulin across skin, as is seen from figure 17. Depending on the choice of data used for evaluation, the lowering of the blood glucose level does not exceed 2 to 5 mg/dl over a period of 30-40 minutes at the most. The effect of a comparable subcutaneous injection is 50 to 200 times higher. Surfactant-containing liposomes, which have not been optimized with regard to their 'transfersomal' properties, are consequently poorly suited for the use as carriers in the case of dermal applications. Surfactant concentration in such carriers thus cannot mediate an optimal agent permeation through skin.

This shows that formulations prepared according to this invention can (still) have a partial activity even if their

content of edge active substances has not been optimized; however, a maximum advantage can only be achieved after the concentration of an edge active substance requiring maximum permeation has been determined and used as described in this patent application.

Possible use of transfersomes for the application of antidiabetics, most notably of insulin, which has been discussed above in examples 166 and 236, will be investigated in more detail in the following text.

Attempts to bring antidiabetic agents into a body without the use of an injection needle have been known for quite some time already (see, for example, the review article by Lassmann-Vague, *Diabete. Metab.* 14, 728, 1989). It has been proposed, for example, to use implantable insulin containers (Wang, P.Y, *Biomaterials* 10. 197, 1989) or pumps (Walter, H et al., *Klin. Wochenschr.* 67, 583, 1989), to administer an insulin solution transnasally (Mishima et al., *J. Pharmacobio.-Dynam.* 12, 31, 1989), perocularly (Chiou et al., *J. Ocul. Pharmacol.* 5, 81, 1989), perorally in a liposomes suspension (Rowland & Woodley, *Biosc. Rep.* 1, 345, 1981) or transrectally; in order to introduce insulin molecules through the skin, a corresponding solution was jet-injected (Siddiqui & Chies, *Crit. Rev. Ther. Drug. Carrier. Syst.* 3, 195, 1987), or brought through the skin with the aid of small injectors (Fiskes, *Lancet* 1, 787, 1989), electric fields (Burnette & Ongpipattanakul, *J. Pharm. Sci.* 76, 765, 1987; Meyer, B.R et al., *Amer. J. Med. Sci.* 297, 321, 1989); chemical additives should also support drug permeation.

All these procedures have hardly brought any real improvements for the therapy of diabetes patients - with the exception of jet injection, perhaps; but the latter is only a very refined, technically extremely complicated form of injection and,

consequently, not very common. The daily therapy of each insulin-dependent patient, consequently, still involves injecting an insulin solution under the skin or into the muscle tissue (De Meijer, P. et al., Neth. J Med. 34, 210. 1989).

Lipids have thus far been discussed as excipients for delayed insulin release in insulin implants (Wang, P.Y Int. J Pharm. 54, 223, 1989); in the form of liposomes they were also suggested for use as vehicles for peroral applications (Patel, 1970), without the therapeutic results really being reproducible, however, (Biochem. Int. 16, 983, 1988). Subsequent publications in the field of insulin containing liposomes, therefore, have dealt with methodological rather than therapeutic issues (Wiessner, J. H. and Hwang, K. J. Biochim. Biophys. Acta 689, 490 1982; Sarrach, D. Stud. Biophys. 100. 95, 1984; Sarrach, D. and Lachmann, U. Pharmazie 40. 642, 1985; Weingarten, C. et al., Int. J. Pharm. 26, 251, 1985; Sammins, M.C. et al., J. Pharm. Sci. 75, 838, 1986; Cervato, G. et al., Chem. Phys. lipids 43, 135, 1987).

According to this invention, the transfersomes described above are used for non-invasive applications of antidiabetic agents, most frequently of insulin, in formulations which were optimized for this purpose.

It is advantageous to use at least one carrier substance for this purpose from the class of physiologically tolerable polar or non-polar lipids or some other pharmacologically acceptable amphiphiles; well-suited molecules are characterized by their ability to form stable agent carrying aggregates. The preferred aggregate form are lipid vesicles, the most preferred membrane structure is a lipid double layer.

It is, furthermore, considered advantageous if at least one

such substance is a lipid or a lipoid from a biological source or some corresponding synthetic lipid; or else, a modification of such lipids, for example a glyceride, glycerophospholipid, sphingolipid, isoprenoidlipid, steroid, sterine or sterol, a sulfur- or carbohydrate-containing lipid, or any other lipid which forms stable double layers; for example, a half-protonated fluid fatty acid. Lipids from eggs, soy-bean, coconuts, olives, safflower, sunflower, linseed, whale oil, Nachtkerze or primrose oil, etc. can be used, for example, with natural, partly or completely hydrogenated or exchanged chains. Particularly frequently, the corresponding phosphatidylcholines are used; as well as phosphatidyl-ethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidic acids and phosphatidylserines, sphingomyelins or sphingophospholipids, glycosphingolipids (e.g. cerebrosides, ceramidpolyhexosides, sulfateids, sphingoplasmalogenes); gangliosides or other glycolipids are also suitable for the use in transfersomes according to this invention. Amongst the synthetic lipids especially the corresponding dioleoyl-, dilinoleyl-, dilinolenyl-, dilinolenoyl-, diaracidonyl-, dimyristoyl-, less frequently dipalmitoyl-, distearoyl-, phospholipide or the corresponding sphingosin derivatives, glycolipids or other diacyl- or dialkyl-lipids are used; arbitrary combinations of the above-mentioned substances are also useful.

It is advantageous if an edge active substance is a nonionic, a zwitterionic, an anionic or a cationic surfactant. It can also contain an alcohol residue; quite suitable components are long-chain fatty acids or fatty alcohols, alkyl-trimethyl-ammonium-salts, alkylsulfate-salts, cholate-, deoxycholate-, glycodeoxycholate-, taurodeoxycholate-salts, dodecyl-dimethyl-aminoxide, decanoyl- or dodecanoyl-N-methylglucamide (MEGA 10, MEGA 12), N-dodecyl-N,N-dimethylglycine, 3-(hexadecyldimethylammonio)-propanesulfonate, N-hexadecyl-

sulfobetaine, nonaethyleneglycol-octylphenylether, nonaethylene-dodecylether, octaethyleneglycol-isotridecylether, octaethylene-dodecylether, polyethylene glycol-20-sorbitane-monolaurate (Tween 20), polyethylene glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-cetylstearylether (Cetomacrogol, Cremophor O, Eumulgin, C 1000) polyhydroxyethylene-4-laurylether (Brij 30), polyhydroxyethylene-23-laurylether (Brij 35), polyhydroxyethylene-8-stearate (Myrj 45, Cremophor AP), polyhydroxyethylene-40-stearate (Myrj 52), polyhydroxyethylene-100-stearate (Myrj 59), polyethoxylated castor oil 40 (Cremophor EL), polyethoxylated hydrated castor oil, sorbitane-monolaurate (Arlacel 20, Span 20), especially preferred decanoyl- or dodecanoyl-N-methylglucamide, lauryl- or oleylsulfate-salts, sodiumdeoxycholate, sodiumglycodeoxycholate, sodiumoleate, sodiumelaidate, sodiumlinoleate, sodiumlaurate, nonaethylene-dodecylether, polyethylene-glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-23-lauryl ether (Brij 35), polyhydroxyethylene-40-stearate (Myrj 52), sorbitane-monolaurate (Arlacel 20, Span 20) etc.

Amongst the most suitable surfactants in these classes of substances are: n-tetradecyl(=myristoyl)-glycero-phosphatidic acid, n-hexadecyl(=palmityl)-glycero-phosphatidic acid, n-octadecyl(=stearyl)-glycero-phosphatidic acid, n-hexadecylene(=palmitoleil)-glycero-phosphatidic acid, n-octadecylene(=oleil)-glycero-phosphatidic acid, n-tetradecyl-glycero-phosphoglycerol, n-hexadecyl-glycero-phosphoglycerol, -n-octadecyl-glycero-phosphoglycerol, n-hexadecylene-glycero-phosphoglycerol, n-octadecylene-glycero-phosphoglycerol, n-tetradecyl-glycero-phosphoserine, n-hexadecyl-glycero-phosphoserine, -n-octadecyl-glycero-phosphoserine, n-hexadecylene-glycero-phosphoserine and n-octadecylene-glycero-phosphoserine.

Total concentration of the basic carrier substance is normally between 0.1 and 30 weight-%; preferably, concentrations between 0.1 and 15 %, most frequently between 5 and 10 % are used.

Total concentration of the edge active substance in the system amounts to 0.1 % through to 99 mol-% of the quantity which is required to solubilize the carrier, depending on each application. Frequently, the optimum is drug dependent - in a concentration range between 1 and 80 mol-%, in particular between 10 and 60 mol-%; most frequently values between 20 and 50 mol-% are favoured.

The concentration of the drug agent in the case of insulin is most frequently in the range between 1 and 500 I.U./ml; concentrations between 20 and 100 I.U./ml are preferred; carrier concentration in the latter case is in the range between 0.1-20 weight-%, frequently between 0.5 and 15 weight-%, most frequently between 2.5 and 10 weight-%.

For preparing a therapeutic formulation, the carrier substances, which are very frequently lipids, are taken as such or dissolved in a physiologically acceptable solvent or a water-miscible solubilizing agent, combined with a polar solution, and made to form carriers.

It is advantageous to use polar solutions containing edge active substances; the latter can also be used with lipids or be contained in a lipid solution.

Carrier formation is preferably initiated by stirring in, by means of evaporation from a reverse phase, by means of an injection or a dialysis procedure, through mechanical agitation, such as shaking, stirring, homogenization,

ultrasonication, friction, shear, freezing-and-thawing, by means of high-and low-pressure filtration, or any other use of energy.

It may be advantageous to incorporate agents only after carrier formation.

If transfersomes are prepared by means of filtration, materials with a pore size of 0.1-0.8 micrometers, very frequently of 0.15-0.3 micrometers, and particularly preferred of 0.22 micrometers are preferably used; several filters can also be used in combination or in a row.

In the case that transfersomes are made by means of ultrasonication, energy densities in the order of 10-50 kW/litre/minute are preferably used; in stirring or rotary machines 1,000 through to 5,000 revolutions per minute are typically used. If high pressure homogenizers are used, pressures in the order of 300-900 Bar normally ensure sufficient transfersome homogeneity and quality after a single passage; in the latter case even suspensions with 20-30 % lipids can be processed without any difficulty.

It is often sensible to prepare transfersomes only shortly before an application from a concentrate or lyophilisate.

Cryopreservatives, such as oligosaccharides, can facilitate the formation of transfersomes from a lyophilisate.

Standard agent, supporting, or additional substances, in particular the stabilizing, protective, gel-forming, appearance-affecting substances and markers can also be used as described in this application.

The following examples illustrate this invention without

implying any limits to its general use. Temperatures are given in degree Celsius, carrier sizes in nanometers, and other quantities in common SI units.

Example 237:

Composition:

120 mg	phosphatidylcholine from soy-bean (purity > 95 %)
20 mg	sodium-cholate p.a. (L/D = 3,2)
150 µl	ethanol (96 %)
1.45 ml	Actrapid HM 100 (recombinant human insulin 100 I.U./ml)

Preparation:

This preparation is produced as described in example 166, with only minor modifications. The main difference is that the lipid/insulin mixture is hand-filtered through a 0.22 µm polycarbonate filter (Sartorius) using a 1 ml injection already few minutes after mixture preparation. The final volume of the suspension is 1.2 ml; the nominal lipid/cholate ratio is 2.8/1, in lipid membranes approx. 2.4/1. The final concentration of insulin is approx. 83 I.U./ml; the vesicle radius one day after preparation is 94 nm on the average; one week later, 170 nm.

Application:

One and half hours after the beginning of the experiment, 240 µl of a sterile suspension of insulin containing transfersomes (with 20 I.U.) were taken. These were applied and uniformly smeared at a dose of approx. 0.7 mg lipid/cm² over the inner side of the right forearm of a male test person starved for 18

hours prior to experiment. 5 minutes later the skin surface is macroscopically dry. Another 45 minutes later no traces of application are visible anymore.

Activity:

At irregular intervals of between 15 and 40 minutes, blood samples are drawn from a soft i.v. catheter placed in the left forearm. The determination of the blood glucose level is performed as described in example 166.

The course in time of the transfersome mediated hypoglycemia is represented in figure 18. The blood glucose level decreases approx. 1.5 hours after drug application by some 10 mg/ml; this artificial hypoglycemia lasts for 4 hours at least and thus attains 70-80 % of the value which can be achieved by a subcutaneous application of a comparable amount of the drug Actrapid. The results of control experiments in which the insulin containing transfersomes are injected subcutaneously are shown as crosses in this figure. The total effect in the latter case is similar to that induced by the free drug injected s.c.

Example 238:

Composition:

216 mg	phosphatidylcholine from soy-bean (487 µl of a 50 % solution in absolute ethanol)
27 mg	phosphatidylglycerol from egg (98 %)
29.45 mg	oleic acid, puriss.
3 ml	Actrapid HM 100 (recombinant human insulin 100 I.U./ml)
40 µl	1 N NaOH
20 µl	1 N NaCl

Preparation:

Lipids are mixed until solution is homogeneously clear. After the addition of an actrapid solution, of alkali and salt solution, an optically opalescent suspension is formed. Filtering of this suspension through a polycarbonate filter with a pore diameter of $0.2\ \mu\text{m}$ yields a much less opalescent suspension which consists of vesicles (transfersomes) with a mean diameter of 320 nm.

Application:

Starting glucose concentration in the blood of a test person (70 kg, 37 years, normoglycemic, starved for 24 hours) is measured over a period of 90 minutes for reference. Subsequently, the above-mentioned transfersome suspension with a nominal concentration of 85 I.U. insulin/ml, which has been aged for 12 hours at 4°C , is applied on the right forearm skin (approx. $330\ \mu\text{l}$ over an area of approx. $15\ \text{cm}^2$); this corresponds to a total applied dose of 28 I.U.

Activity:

Blood specimens are collected through a heparinized, permanent, soft catheter placed in a vein in the left forearm; 0.5 ml of each sample are sedimented and immediately frozen for further use. The remaining volume is used for the in situ determination of the blood glucose concentration by an enzymatic method. The measured glucose concentration decreases by approx. 8 mg/dl after approx. 2.5 hours and remains diminished for more than 4.4 hours. This corresponds to 75 % of the maximally achievable effect, as concluded from control experiments performed by injecting insulin s.c. The pharmacokinetics of this experimental series is represented in

figure 19.

Figure 20 gives the results of three typical experiments with insulin. They illustrate the results obtained by one percutaneous and two s.c. drug applications.

Example 239:

Composition:

143 mg	phosphatidylcholine from soy-bean
18 mg	phosphatidylglycerol from egg (98 %)
19.6 mg	oleic acid, puriss.
2 ml	Actrapid HM 100 (200 I.U.)
25 μ l	1 N NaOH

Preparation:

Lipids are weighed into a glass vial and mixed with a standard insulin solution. The resulting opaque suspension is ultrasonicated directly, using a titanium probe-tip (approx. 5 W, 3x5 seconds at 22°C in 60 seconds intervals). The resulting, optically clear but still opalescent suspension contains vesicles with a mean radius of 114 ± 17 nm.

Application and Activity:

The results of this test series are within the limits of experimental error identical to those obtained in example 238.

Example 240:

Composition:

143 mg	phosphatidylcholine from soy-bean
--------	-----------------------------------

18 mg	phosphatidylglycerol from egg (98 %)
20.5 mg	sodium oleate
2 ml	Actrapid HM 100 (200 I.U.)

Preparation:

The lipids are dissolved in a glass vial in 0.15 ml abs. ethanol and then combined with a standard insulin solution. Further procedure is as described in example 239.

Application and Activity:

Over an area of approx. 5 cm² on the forearm skin of a test person a piece of fine-mesh synthetic cloth is fixed. This is then covered with 350 µl of an insulin containing transfersome suspension and left uncovered to dry.

The resulting decrease of the blood glucose level after 4 hours amounts to 7.8 mg/dl and after 6 hours to 8.5 mg/dl. It is thus comparable to the result obtained in experiment no. 238.

Example 241:

The procedure is at first as described in example 238 except that no salt solution is added to the sample suspension; the opaque crude transfersome suspension is divided into two parts. One of these consisting of 50 % of the total volume is passed through a sterile filter; the other half is ultrasonicated for 15 seconds at room temperature at a power of approx. 5 W. The mean diameter of carriers in both halves is similar, 300 nm or 240 nm, respectively.

Example 242:

The procedure is as described in examples 238 and 240. Transfersomes, however, are filtered one, two and three times in a row. The mean vesicle diameter in the resulting three samples are 300, 240, and 200 nm, resp..

The transfersomes of examples 241 and 242 yield similar hypoglycemic results in biological tests as those of example 238.

Example 243:

Composition:

144,9;152 mg	phosphatidylcholine from soy-bean
24.8;17.6 mg	desoxycholate, Na-salt
1.45;1.55 ml	Actrapid HM 100 (145 I.U.)
0.16 ml	ethanol, absolute

Preparation:

Lipids are weighed into glass vials, dissolved with ethanol and mixed with an insulin solution. The resulting opaque suspension is aged over night and subsequently filtered through a 0.22 micrometer filter at t=12 hours. The nominal insulin concentration is 83 or 84 I.U; the mean vesicle radius in both cases is 112 nm.

Application and Activity:

General experimental conditions are as described in examples 237-239. Transfersome suspensions (0.36 ml, corresponds to 30 I.U.) are applied onto the inner side of a forearm skin in both cases; the blood samples are taken from a soft catheter placed in a vein in the other forearm.

The results of these two experiments are given in figure 21. They show that preparations with a relatively high surfactant concentration (Sample 1, L/S=3/1) can cause a hardly significant decrease in the blood glucose level; transfersomes close to their optimum, however, with a surfactant concentration lower by approx. 30 % (L/S=4.5/1), cause a very pronounced 'hypoglycemia' which lasts for many hours.

This is another proof that the transfersomes tend to transport drugs through intact skin according to a completely new principle of action which is dissimilar to that of classical pharmaceutical formulations.

This example, in addition to example 236, furthermore, suggests the following conclusion: for the systems investigated, also surfactant concentrations can be used which are remote from the transfersomal optimum (without the carrier activity being lost completely); notwithstanding this, particularly advantageous results are obtained when the surfactant concentration has been determined and chosen to be in a range which ensures maximum carrier elasticity and thus permeation capability of the transfersomes in combination with sufficiently high carrier stability to dissolution, bursting, agent loss, etc.

Claims:

1. Preparation for the application of agents in the form of minute droplets of fluid, in particular with a membrane-like coating consisting of one or several layers of amphiphilic molecules or of one amphiphilic carrier substance, for transporting agents into and through natural barriers and constrictions such as skin and similar materials, characterized by the fact that each preparation contains an edge active substance at a concentration which amounts up to 99 mol-% of the concentration of this substance required to solubilize the droplet.
2. Preparation according to claim 1, wherein the concentration of edge active substance amounts to at least 0.1 mol-%, in particular between 1 and 80 mol-%, preferably between 10 and 60 mol-%, and particularly preferred between 20 and 50 mol-% of the solubilization-inducing concentration of edge active substances, whereby the edge activity of a droplet unit is preferably close to approx. 10 Piconewton or less.
3. Preparation according to claims 1 or 2, characterized by the fact that the preparation contains an amount of an amphiphilic substance as a carrier or as a basis for the membrane-like envelope of the droplet forming hydrophilic fluid, the agent being contained in the carrier substance, in the shell, and/or in the droplet material itself.
4. Preparation as claimed in claim 3, wherein said amphiphilic substance is a lipid-like material and said edge active substance is preferably a surfactant.

5. Preparation as claimed in one of claims 1 through 4, wherein the content of said amphiphilic substance for the applications on human or animal skin amounts to 0.01 through 30 weight-% of the preparation mass, preferably between 0.1 and 15 weight-% and particularly preferred between 5 and 10 weight-%.
6. Preparation as claimed in one of claims 1 through 4, wherein the content of the amphiphilic substance in the formulation for application on plants is 0.000001 through 10 weight-%, preferably between 0.001 and 1 weight-% and particularly preferred between 0.01 and 0.1 weight-%.
7. Preparation as claimed in any one of the preceding claims, wherein an agent is an adrenocorticostatic, a β -adrenolytic, an androgen or antiandrogen, antiparasitic, anabolic, anaesthetic or analgesic, analeptic, antiallergic, antiarrhythmic, antiarterosclerotic, antiasthmatic and/or bronchospasmolytic, antibiotic, antidrepressant and/or antipsychotic, antidiabetic, an antidote, antiemetic, antiepileptic, antifibrinolytic, anticonvulsive, an anticholinergic, an enzyme, coenzyme or a corresponding inhibitor, an antihistaminic, antihypertonic, a biological inhibitor of drug activity, an antihypotonic, anticoagulant, antimycotic, antimyasthenic, an agent against Morbus Parkinson, an antiphlogistic, antipyretic, antirheumatic, antiseptic, a respiratory analeptic or a respiratory stimulant, a broncholytic, cardiotonic, chemotherapeutic, a coronary dilatator, a cytostatic, a diuretic, a ganglium-blocker, a glucocorticoid, an antiflow agent, a haemostatic, hypnotic, an immunoglobuline or its fragment or any other immunologically active substance, a bioactive carbohydrate (derivative), a contraceptive, an anti-migraine agent, a mineralcorticoid, a morphine-

antagonist, a muscle relaxant, a narcotic, a neuraltherapeutic, a nucleotide, a neuroleptic, a neurotransmitter or some of its antagonists, a peptide(derivative), an opthalmic, (para)-sympaticomimetic or (para)sympathicolytic, a protein(derivative), a psoriasis/neurodermitis drug, a mydriatic, a psychostimulant, rhinologic, any sleep-inducing agent or its antagonist, a sedating agent, a spasmolytic, tuberlostatic, urologic, a vasoconstrictor or vasodilatator, a virustatic or any wound-healing substance, or several such agents.

8. Preparation as claimed in one of claims 1 through 6, wherein said agent is a growth modulating substance for living organisms.
9. Preparation as claimed in one of claims 1 through 6, wherein said agent exerts some biocidal activity and particularly is an insecticide, a pesticide, a herbicide or a fungicide.
10. Preparation as claimed in one of claims 1 through 6, wherein an agent is an attractant, in particular from the class of pheromones.
11. A method for manufacturing preparations for the application of agents in the form of minute droplets of a fluid, in particular in a membrane-like 'envelope' consisting of one or several layers of amphiphilic molecules, or supplemented with an amphiphilic carrier substance, in particular for the transport of agents in and through natural barriers and constrictions, such as skin and the like, characterized by the fact that the concentration of an edge active substance required for the solubilization of a carrier entity is determined and

then an amount of the edge active substance which is close to the former concentration but still guarantees a sufficient carrier stability and permeation capability is used for the preparation.

12. Method as claimed in claim 11, wherein the stability and the permeation capacity of the fluid 'droplet' are determined by means of filtration, if required under pressure, through a fine-pore filter or by means of any other controlled mechanical fragmentation.
13. Method as claimed in claims 11 or 12, wherein the content of said edge active substance is between 0.1 and 99 mol-%, and in particular between 1 and 80 mol-%, preferably between 10 and 60 mol-% and most preferred between 20 and 50 mol-% of the concentration at which solubilization of the carrier is achieved.
14. Method as claimed in one of claims 11 through 13, wherein said mixture of substances required for the formation of a preparation is subjected to filtration, ultrasonication, stirring, agitating or any other mechanical fragmentation.
15. Preparation as claimed in one of claims 1 through 10, wherein said preparation for non-invasive application contains at least one antidiabetic agent, in particular insulin.
16. Preparation as claimed in claim 15, characterized by the fact that it contains a physiologically compatible polar or non-polar lipid as an amphiphilic carrier substance, the carrier membrane preferably having a double layer structure.

17. Preparation as claimed in claim 16, wherein the amphiphilic substance is a lipid or a lipoid from any biological source or a corresponding synthetic lipid, or else comprises a modification of such lipids, a glyceride, in particular glycerophospholipid, isoprenoidlipid, sphingolipid, steroid, sterin or sterol, a sulfur- or carbohydrate-containing lipid, or else any other lipid which forms stable double layers, preferably a half-protonated fluid fatty acid, and preferably a phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, a phosphatidic acid, a phosphatidylserin, a sphingomyelin or sphingophospholipid, glycosphingolipid (e.g. cerebroside, ceramidepolyhexoside, sulfatide, sphingoplasmalogene), a ganglioside or any other glycolipid or a synthetic lipid, preferably a dioleoyl-, dilinoleyl-, dilinolenyl-, dilinolenoyl-, diarachidoyl-, dimyristoyl-, dipalmitoyl, distearoyl, phospholipid or corresponding sphingosinderivative, a glycolipid or any other diacyl- or dialkyl-lipid.
18. Preparation as claimed in one of claims 15 through 17, containing several edge active substances.
19. Preparation as claimed in one of claims 15 through 18, wherein said edge active substance is a nonionic, a zwitterionic, an anionic or a cationic surfactant, in particular a long-chain fatty acid or a long-chain fatty alcohol, an alkyl-trimethyl-ammonium-salt, alkylsulfate-salt, cholate-, deoxycholate-, glycodeoxycholate-, taurodeoxycholate-salt, dodecyl- dimethyl-aminoxide, decanoyl- or dodecanoyl-N- methylglucamide (MEGA 10, MEGA 12), N-dodecyl-N,N- dimethylglycine, 3-

(hexadecyldimethylammonio)-propane-sulfonate, N-hexadecyl-sulfobetaine, nonaethylene-glycol-octylphenylether, nonaethylene-dodecylether, octaethyleneglycol-isotridecylether, octaethylene-dodecylether, polyethylene glycol-20-sorbitane-monolaurate (Tween 20), polyethylene glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-cetylstearyl ether (Cotomacrogol, Cremophor O, Eumulgin, C 1000) polyhydroxyethylene-4-laurylether (Brij 30), polyhydroxyethylene-23-laurylether (Brij 35), polyhydroxyethylene-8-stearate (Myrj 45, Cremophor AP), polyhydroxyethylene-40-stearate (Myrj 52), polyhydroxyethylene-100-stearate (Myrj 59), polyethoxylated castor oil 40 (Cremophor EL), polyethoxylated hydrated castor oil, sorbitane-monolaurate (Arlacel 20, Span 20), particularly preferred decanoyl- or dodecanoyl-N-methylglucamide, lauryl- or oleoylsulfate-salts, sodiumdeoxycholate, sodiumglycodeoxycholate, sodiumoleate, sodiumelaidate, sodiumlinoleate, sodiumlaurate, nonaethylene-dodecylether, polyethylene glycol-20-sorbitane-monooleate (Tween 80), polyhydroxyethylene-23-laurylether (Brij 35), polyhydroxyethylene-40-stearate (Myrj 52) and/or sorbitane-monolaurate (Arlacel 20, Span 20) and lysophospholipid, such as n-octadecylen(=oleoyl)-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-dilauryl-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-tetradecyl-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine and corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipids.

20. Preparation as claimed in one of claims 15 through 19, characterized by the fact that it contains 1 through 500 I.U. insulin/ml as agent, preferably between 20 and 100

I.U. insulin/ml and the concentration of the carrier substance in the preparation is in the range of 0.1 through 20 weight-%, in particular between 0.5 and 15 weight-%, particularly preferred between 2.5 and 10 weight-%.

21. Preparation as claimed in one of claims 15 through 20, characterized by the fact that a phosphatidylcholine and/or a phosphatidylglycol is used as an amphiphilic substance, and that a lysophosphatidic acid or lysophosphoglycerol, a deoxycholate-, glycodeoxycholate- or cholate salt, a laurate, myristate, oleate, palmitoleate, or a corresponding phosphate- or sulfate-salt, and/or a Tween- or a Myrj-surfactant is used as an edge active substance, recombinant human insulin being the preferred agent.
22. Preparation as claimed in one of claims 15 through 21, wherein the radius of said vesicular droplets in a preparation is between approx. 50 and approx. 200 nm, preferably between approx. 100 and 180 nm.
23. A method for the preparation of a formulation for the non-invasive application of antidiabetic agents, wherein said liposome-like droplets are formed from at least one amphiphilic substance, at least one hydrophilic fluid, at least one edge active substance, and at least one antidiabetic agent which together form the preparation.
24. Method as claimed in claim 23, wherein the edge active substance and the amphiphilic substance, and the hydrophilic substance and the agent are separately mixed together and, if required, dissolved in a solution, the resulting mixtures or solutions then being combined

as one mixture to induce the formation of carrier particles, particularly by action of mechanical energy.

25. Method as claimed in claims 23 or 24, wherein said amphiphilic substance is either used as such or dissolved in a physiologically compatible solvent which is very frequently miscible with hydrophilic fluids, in particular water, or in a solvation mediating agent together with a polar solution.
26. Method as claimed in claim 25, wherein the polar solution contains at least one edge active substance.
27. Method as claimed in one of claims 23 through 26, characterized by the fact that the formation of droplets is induced by substance addition into a fluid phase, evaporation from a reverse phase, using an injection- or dialysis procedure, with the aid of mechanical stress such as shaking, stirring, homogenizing, ultrasonication, shear, freezing and thawing, or high- or low-pressure filtration.
28. Method as claimed in claim 27, characterized by the fact that the formation of droplets is caused by filtration the filtering material having pore diameters of 0.1 through 0.8 μm , in particular with 0.15 through 0.3 μm , especially preferred 0.22 μm , several filters being sometimes used in a sequence.
29. Method as claimed in one of claims 23 through 28, wherein inclusion of said agents occurs at least partly after the droplet formation.
30. Method as claimed in one of claims 23 through 29, wherein liposome-like droplets are prepared just before

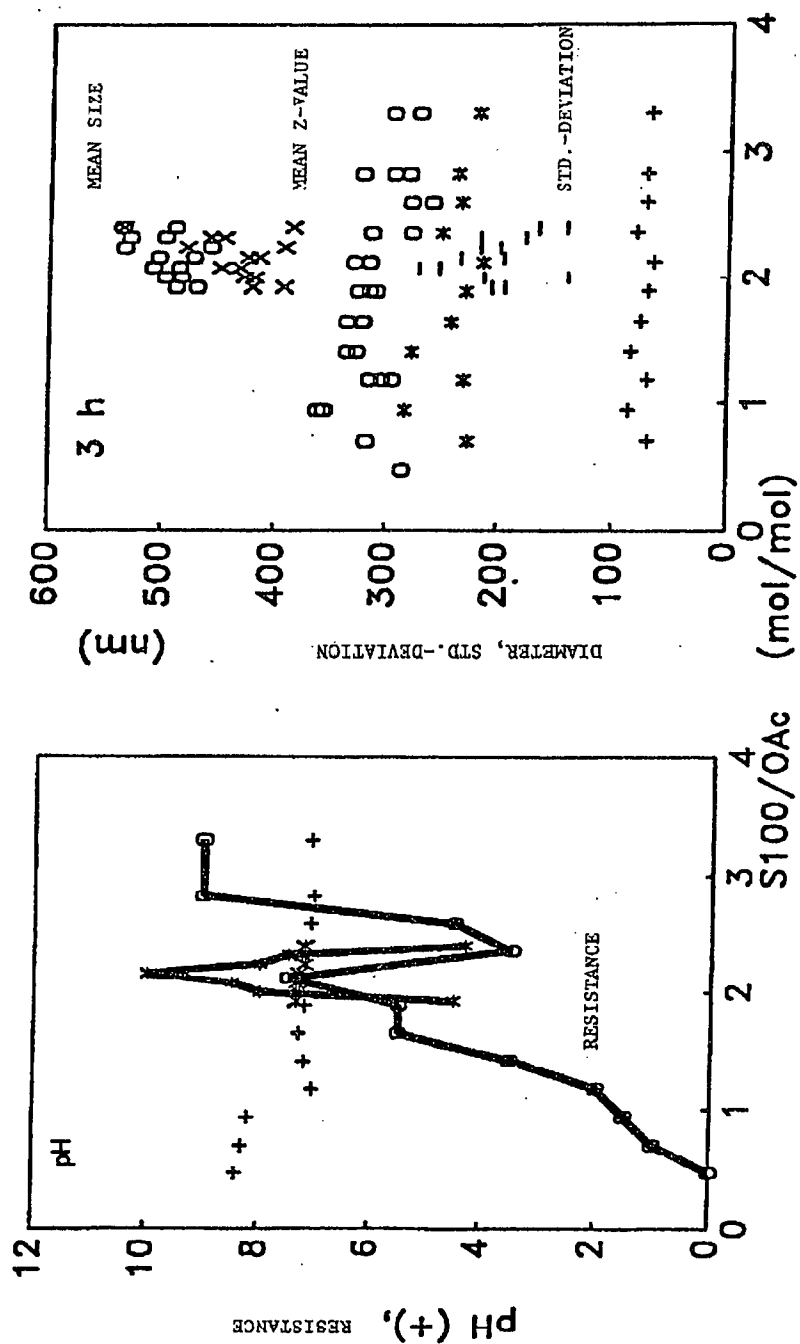
2067754

- 158 -

their application from a suitable concentrate or a
lyophilisate.

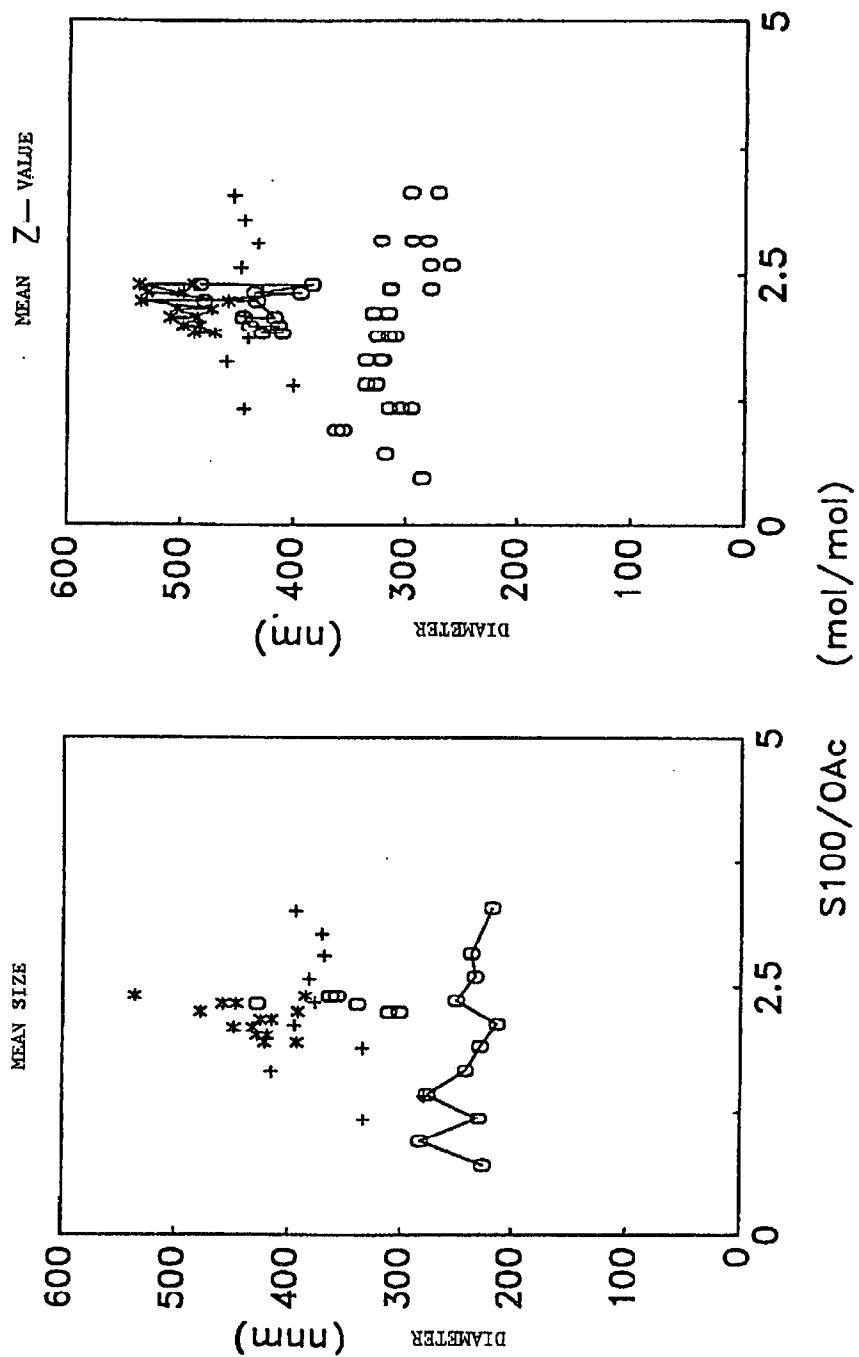
2067754

FIGURE 1

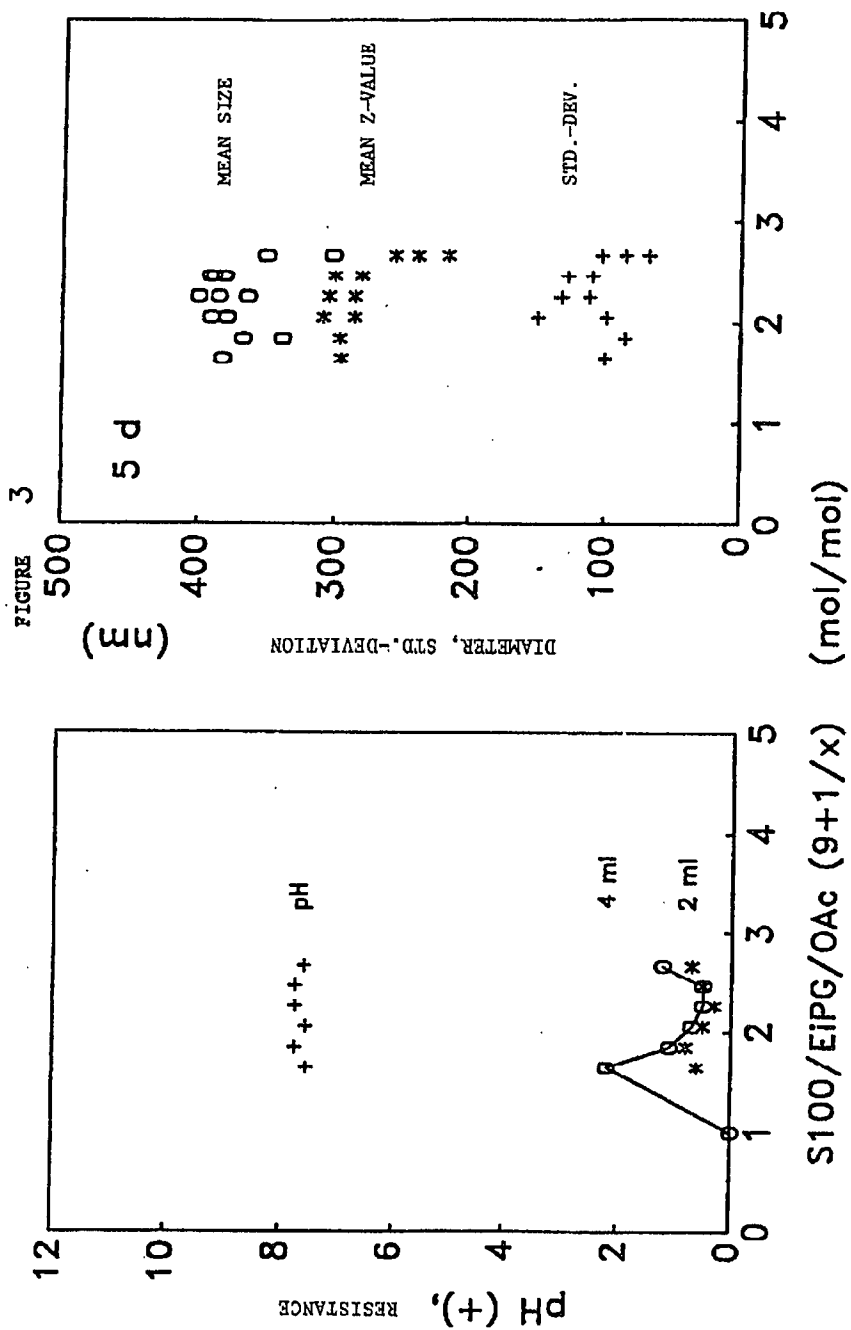


2067754

FIGURE 2

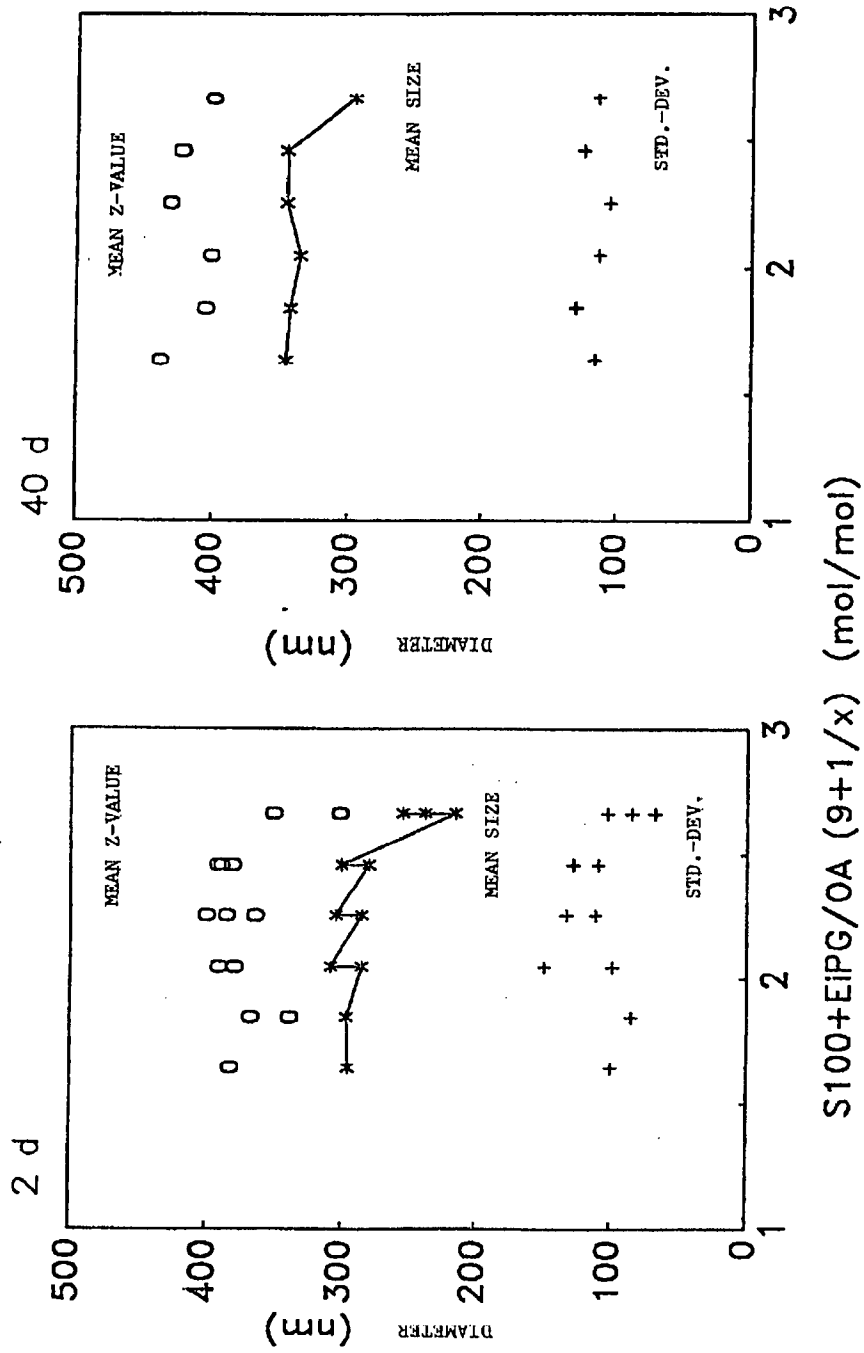


2067754



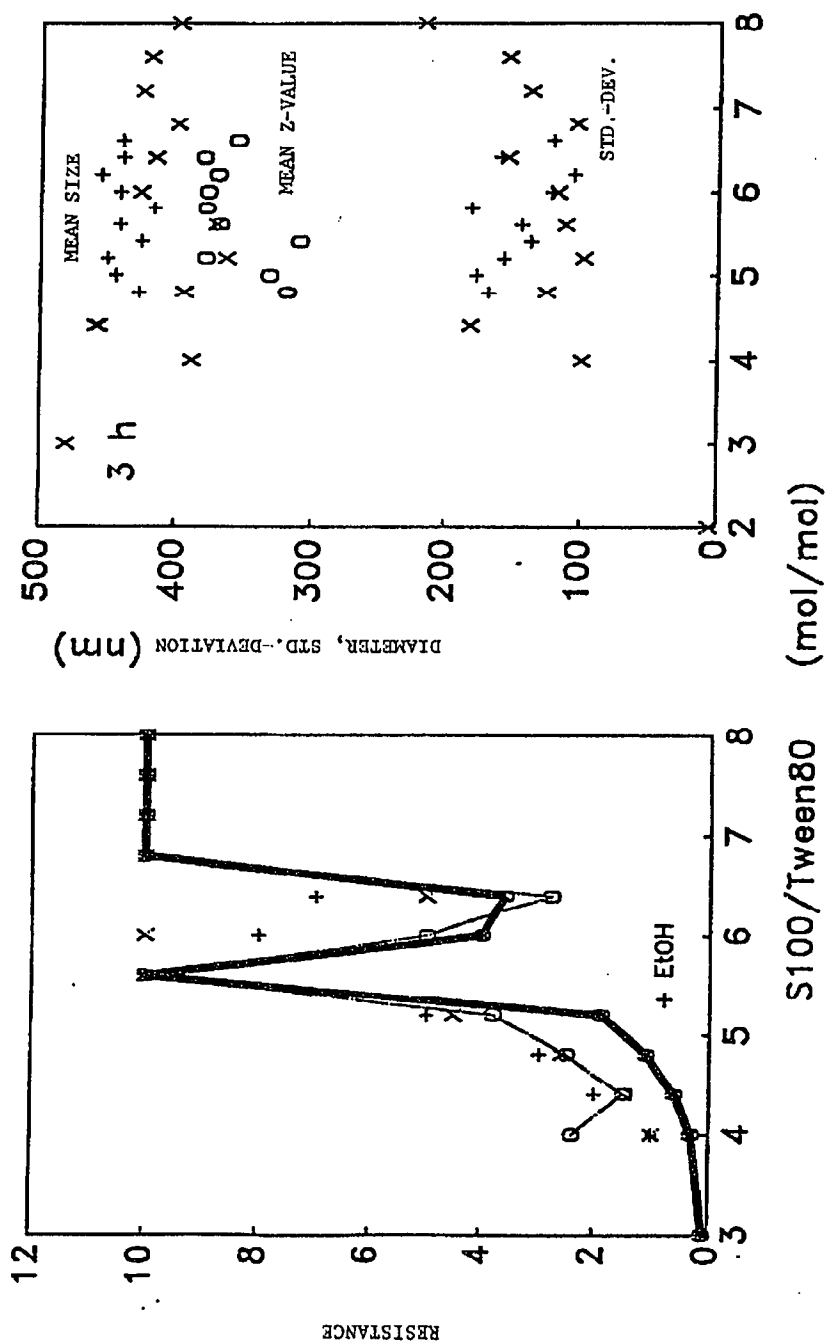
2067754

FIGURE 4



2067754

FIGURE 5



2067754

FIGURE 6

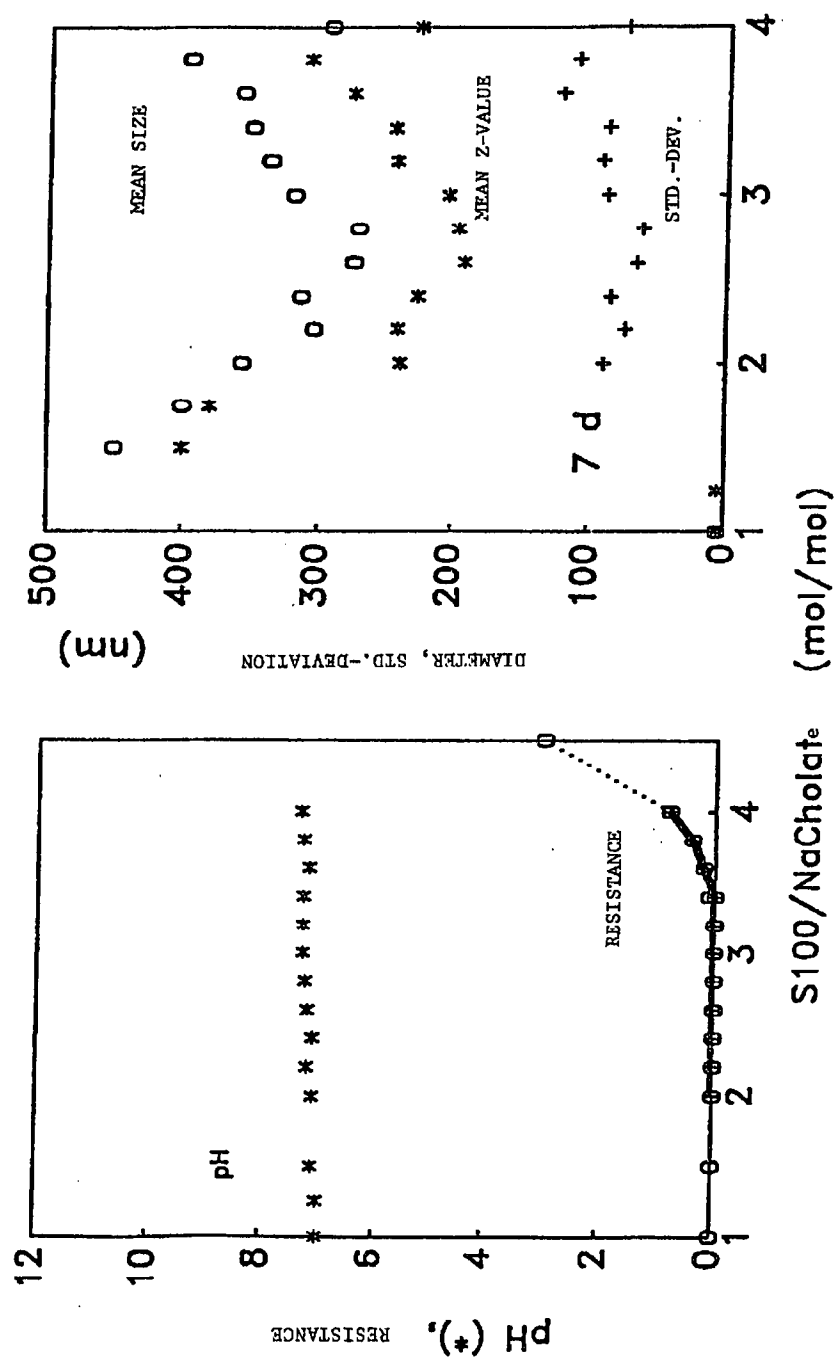
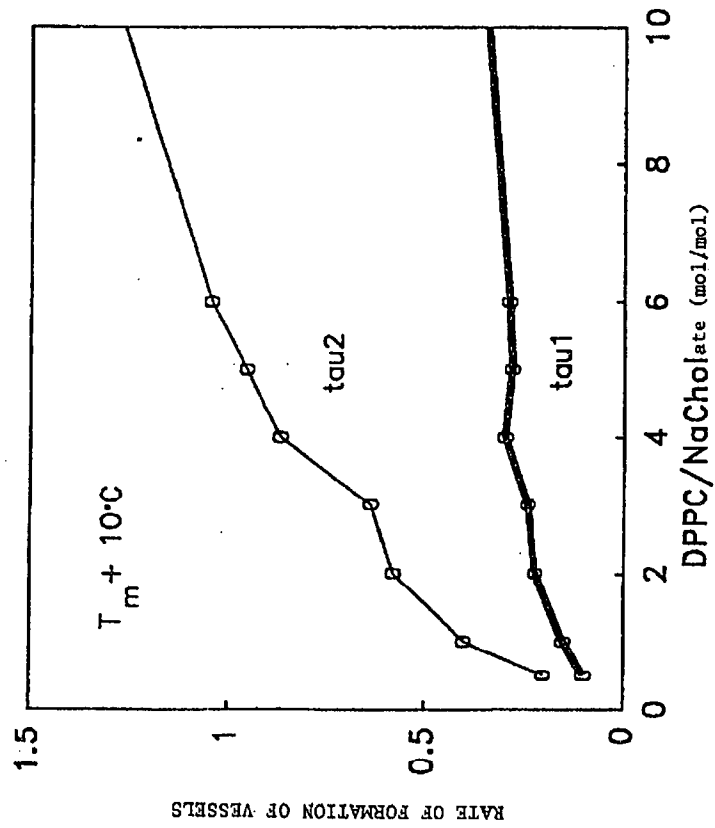
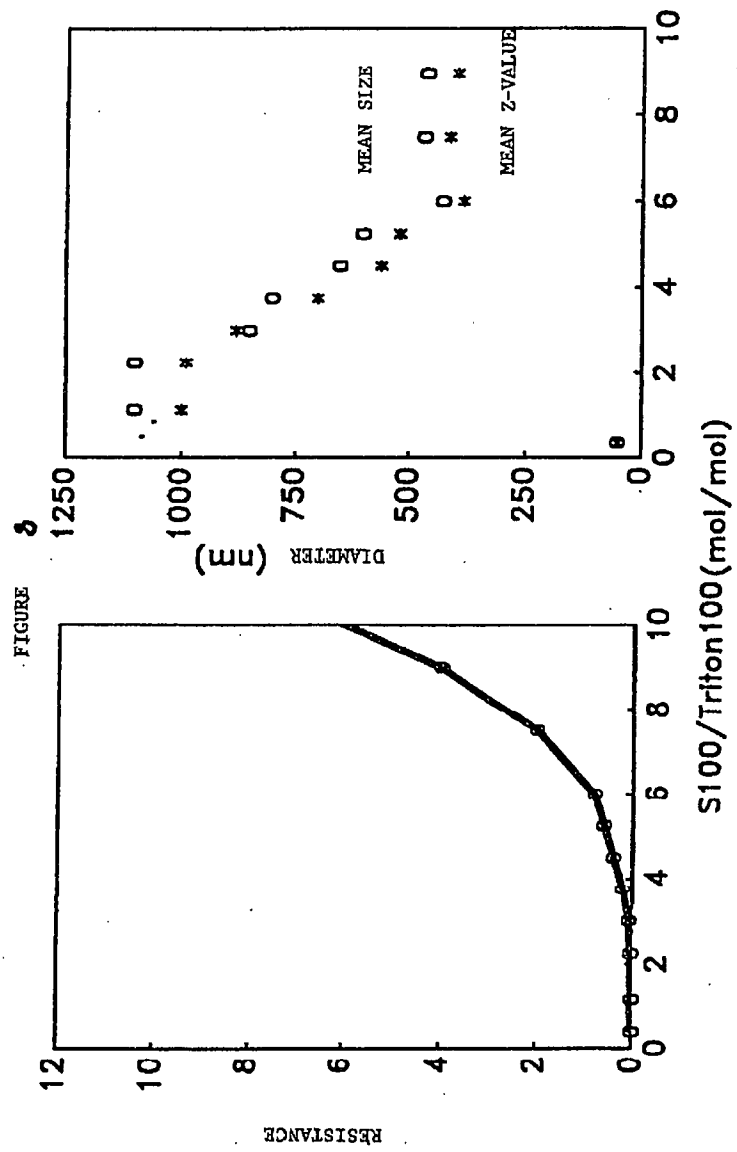
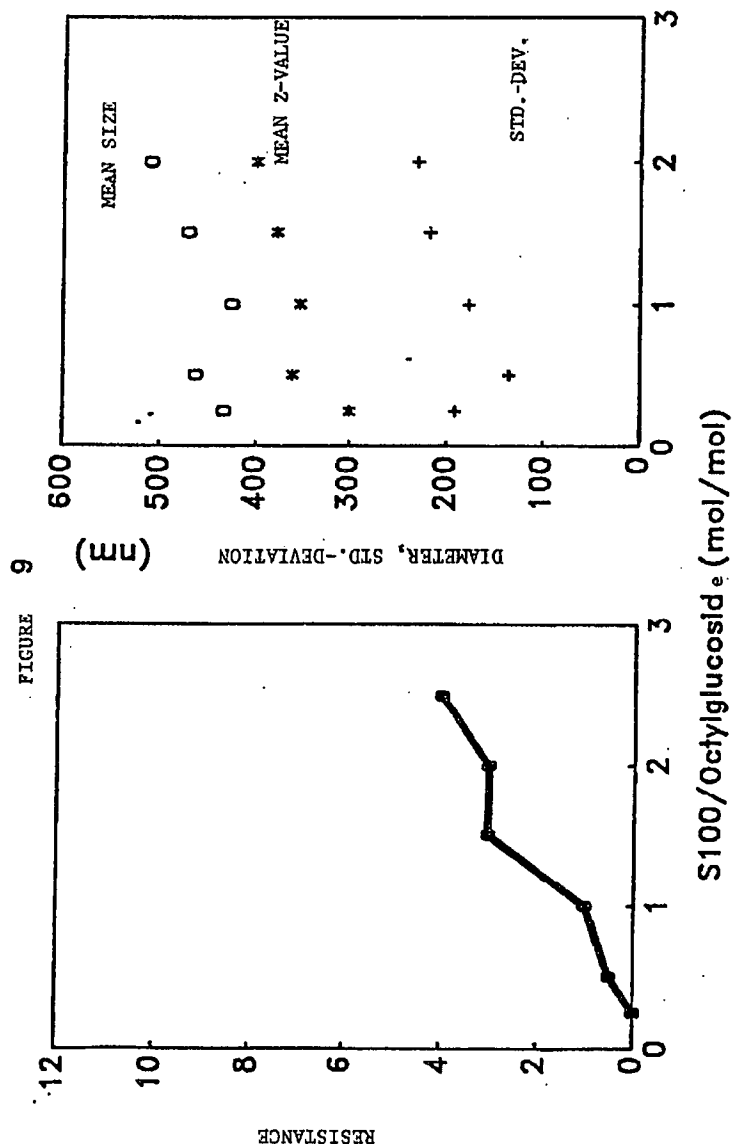


FIGURE 7



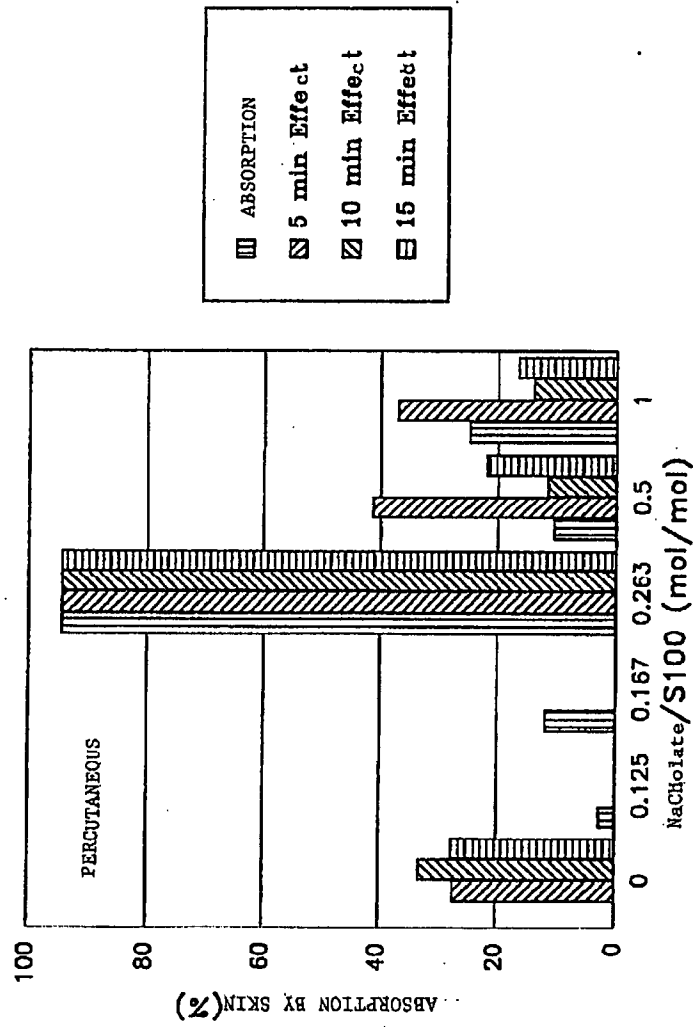


2067754

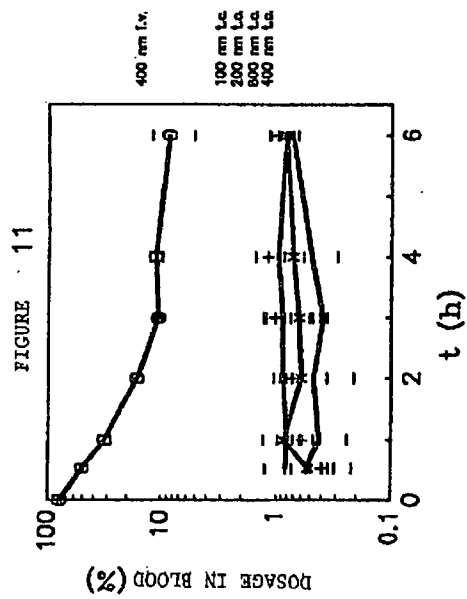


2067754

FIGURE 10



2067754



2067754

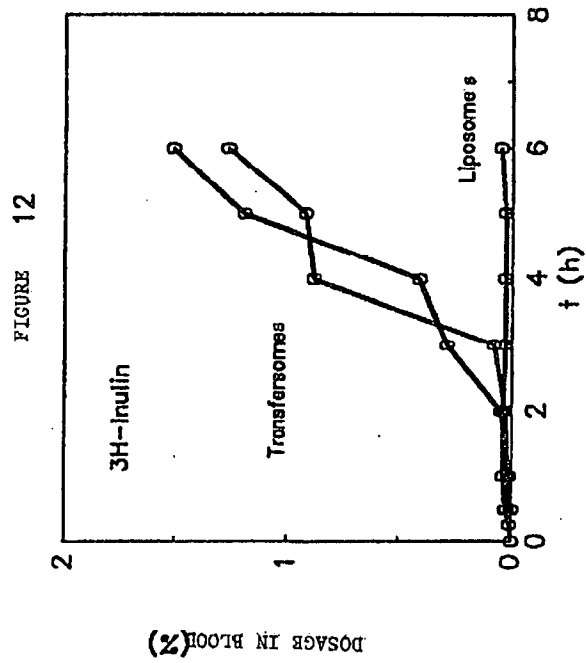
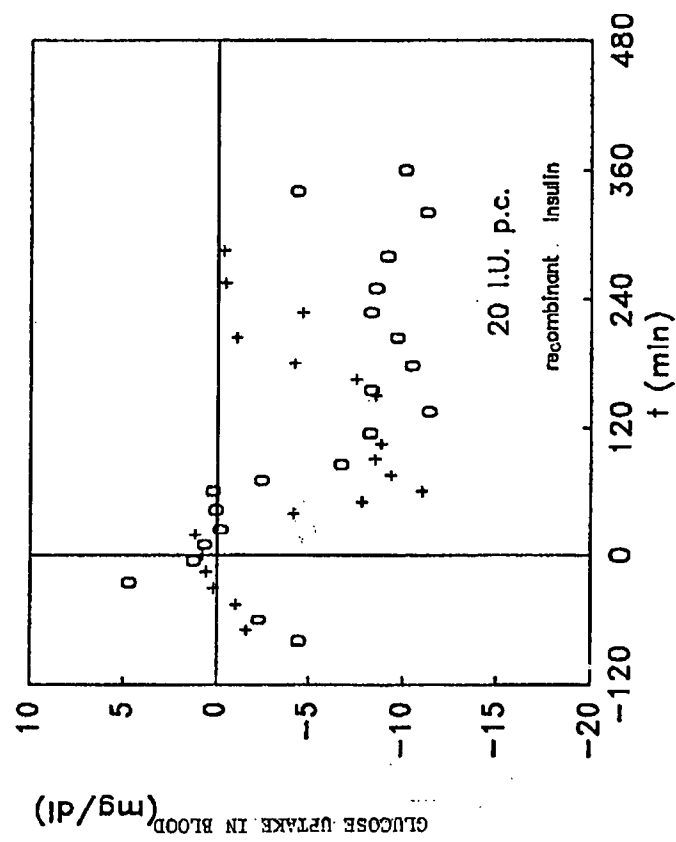
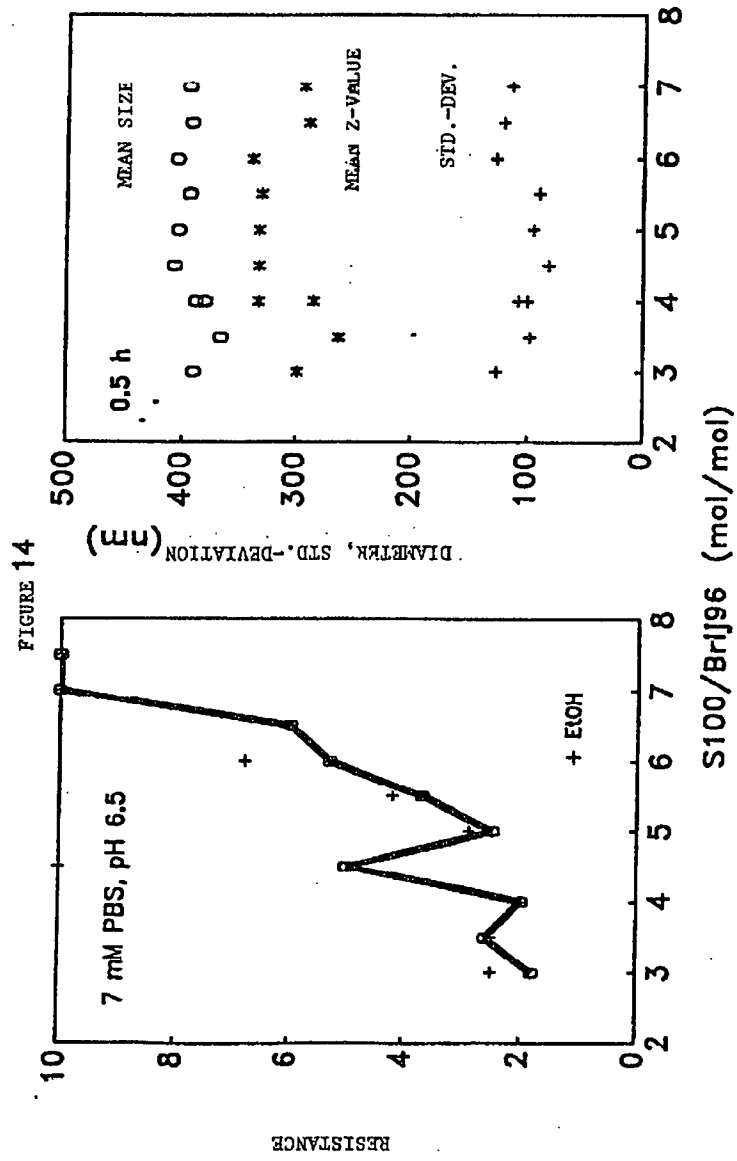


FIGURE 13





2067754

FIGURE 15

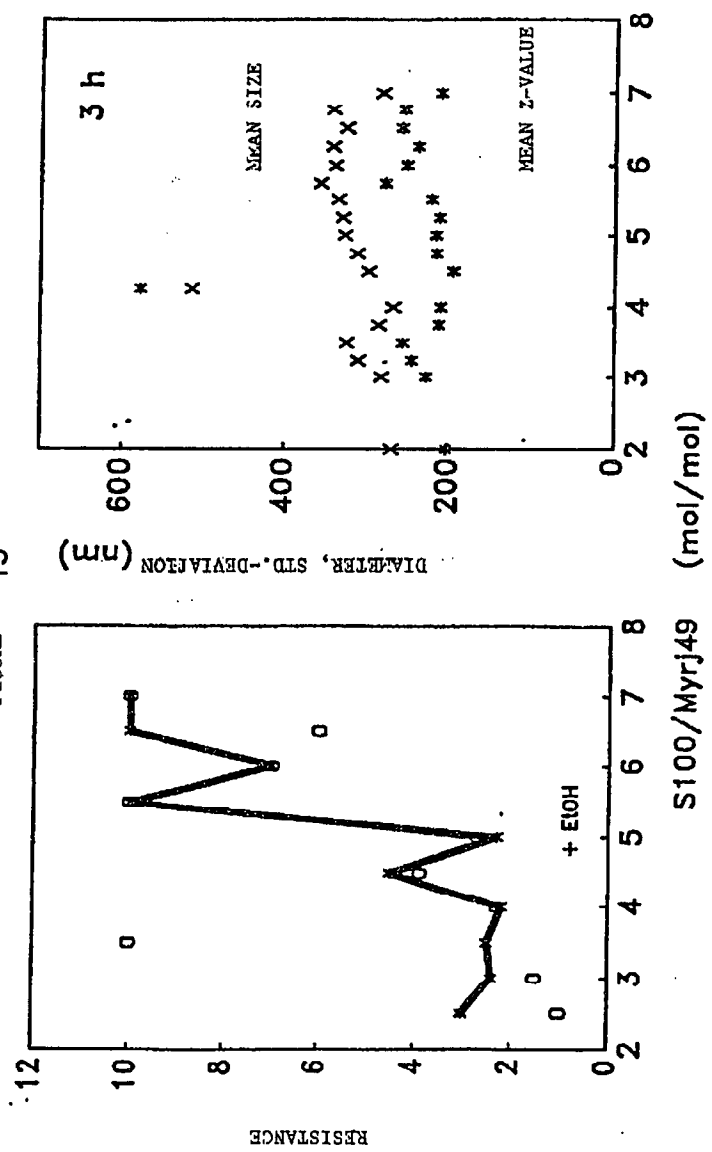
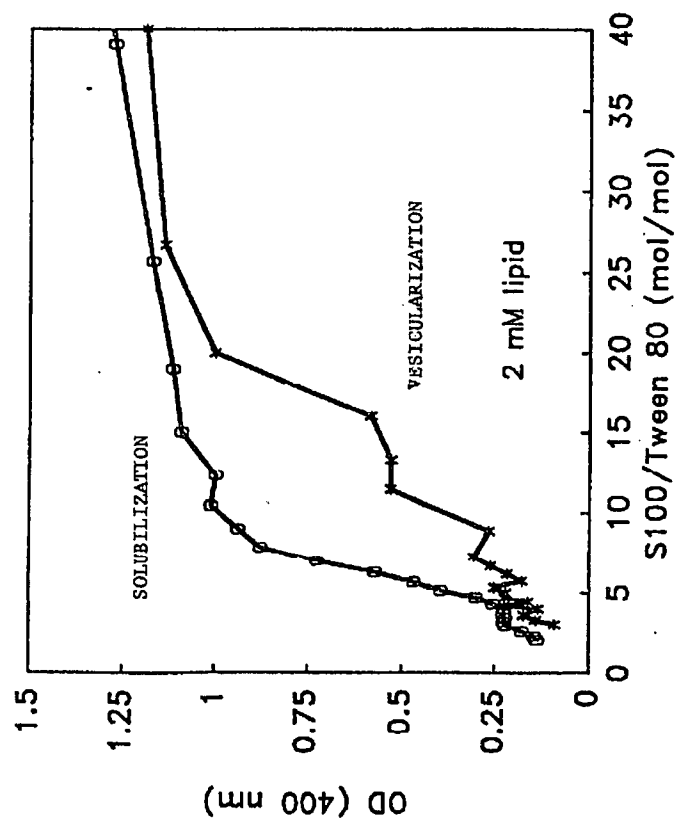


FIGURE 16



2067754

FIGURE 17

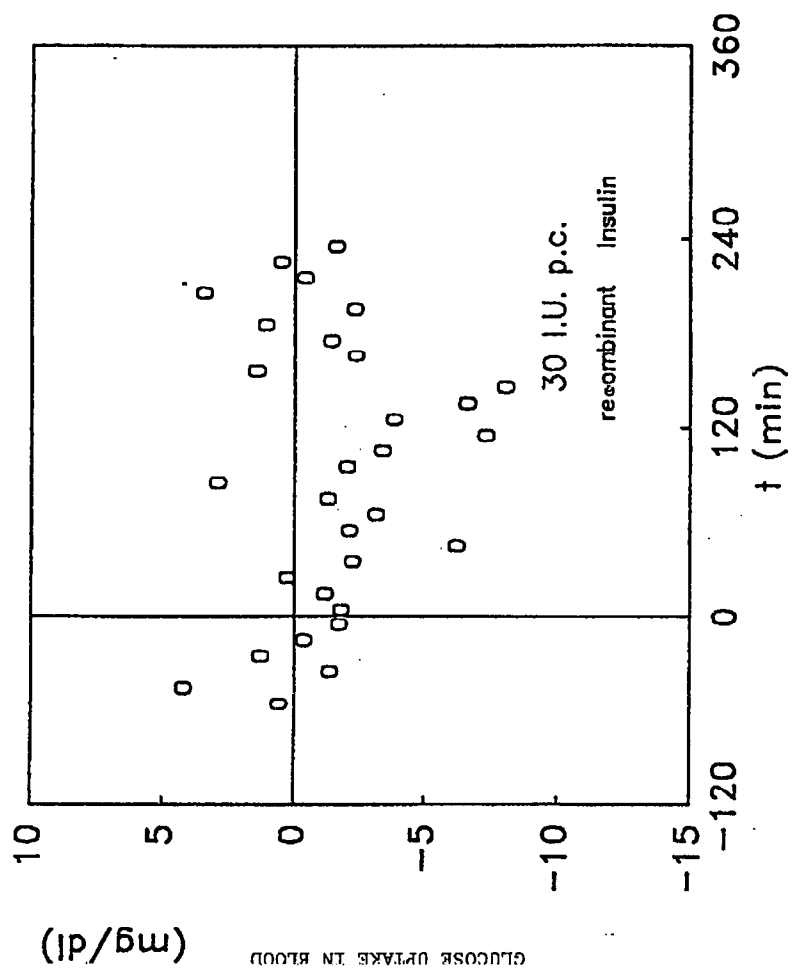


FIGURE 18

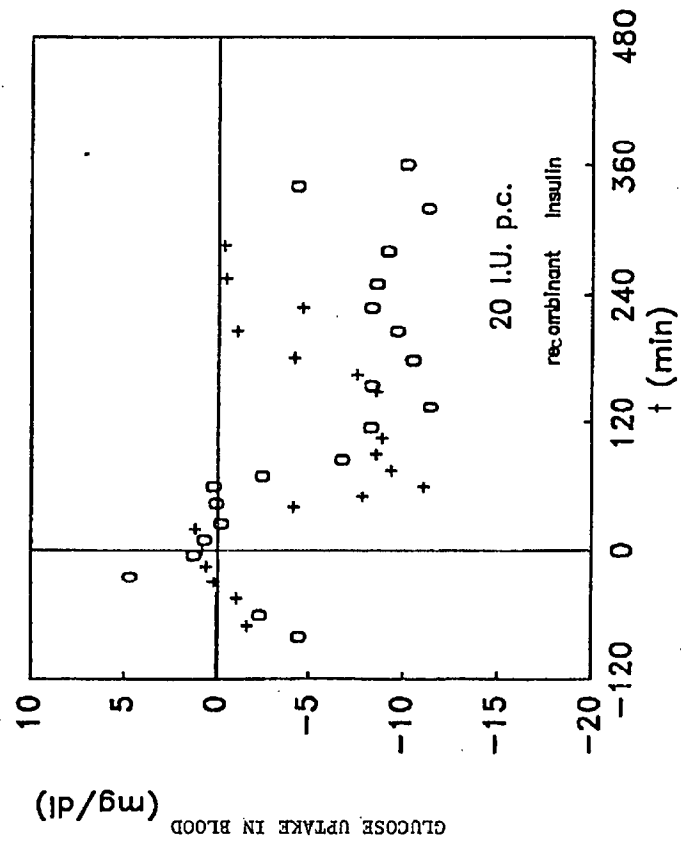
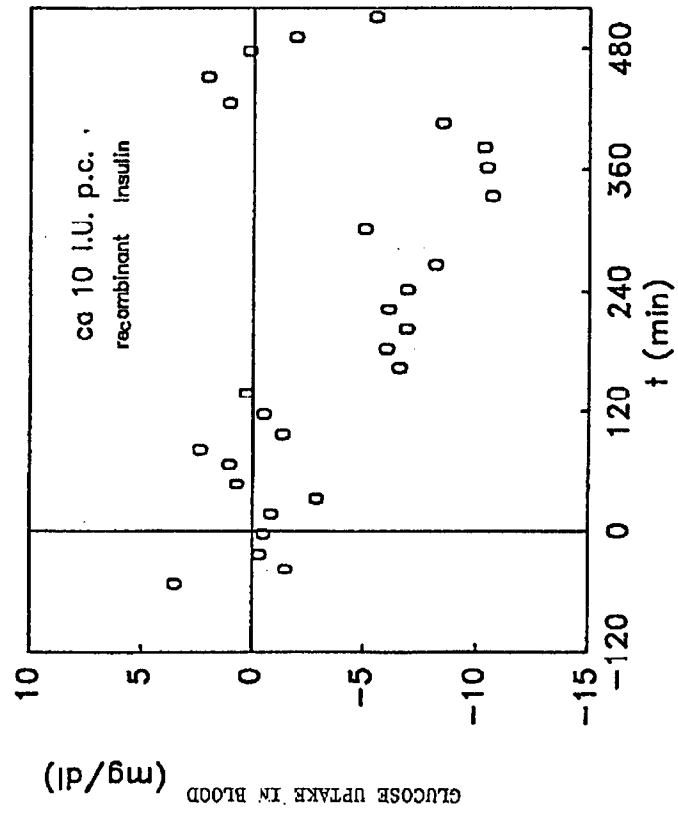


FIGURE 19



2067754

FIGURE 20

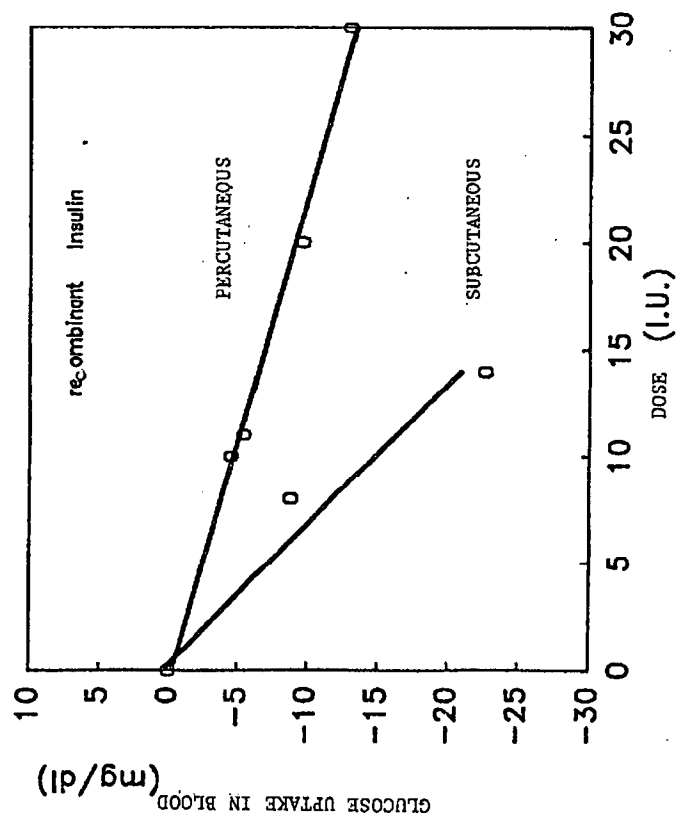
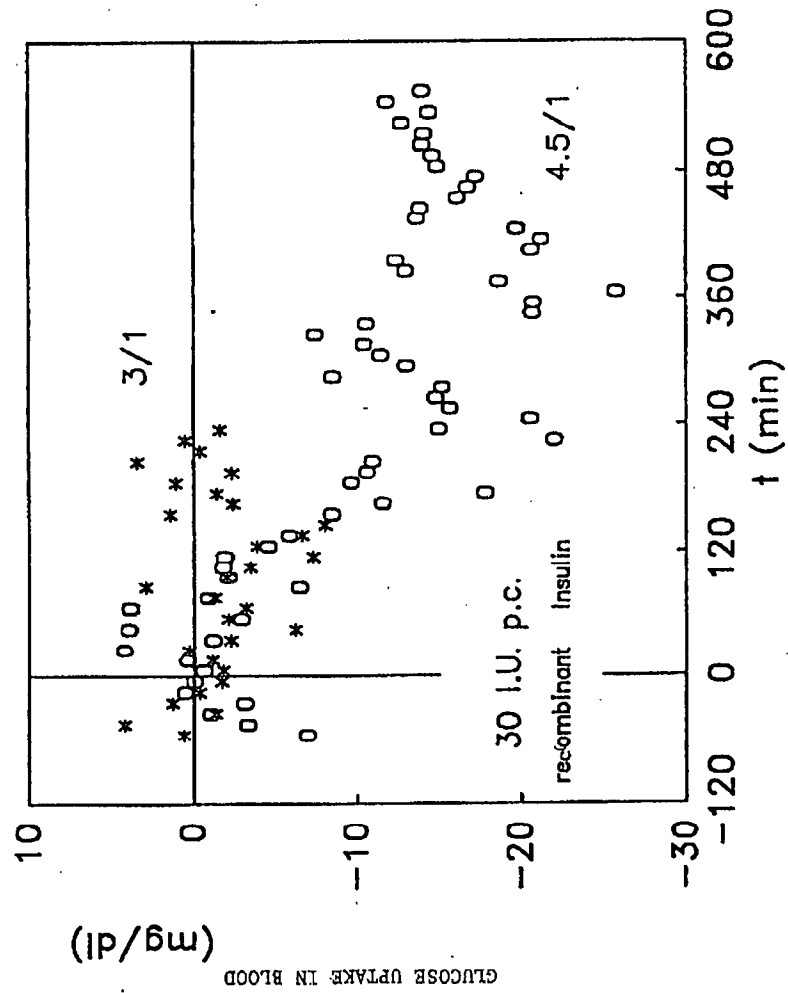


FIGURE 21





Consommation
et Corporations Canada

Consumer and
Corporate Affairs Canada

Bureau des brevets

Patent Office

Ottawa, Canada
K1A 0C9

(11) (C) **1,289,420**

(21) 561,288

(22) 1988/03/11

(45) 1991/09/24

(52) 117-46.7

C.L. CR. 167-163

(51) INTL.CL. ⁵ A61K-9/50; B01J-13/02; B01J-13/08

(19) (CA) **CANADIAN PATENT** (12)

(54) **Method of Producing High Aqueous Volume Multilamellar Vesicles**

(72) Wallach, Donald F.H. , U.S.A.

(73) Micro-Pak, Inc. , U.S.A.

(30) (US) U.S.A. 025,525 1987/03/13
(US) U.S.A. 078,658 1987/07/28

(57) 34 Claims

NO DRAWING

Canada

561288

ABSTRACT

Disclosed is a new method of producing high aqueous volume multilamellar lipid vesicles. The method uses less expensive materials than those commonly used, is faster than classical methods, and produces vesicles with a much higher encapsulated mass and captured volume than was previously available.

1 Background of the Invention

 Liposomes or lipid vesicles have been known
since at least 1965. There are three general types
of liposomes: multilamellar vesicles (MLV),
5 onion-like structures having a series of
substantially spherical shells formed of lipid
bilayers interspersed with aqueous layers, ranging in
diameter from about 0.1 - 4 μ m; large (greater than
1 μ m diameter) unilamellar vesicles (LUV) which
10 have a lipid bilayer surrounding a large,
unstructured aqueous phase; and small unilamellar
vesicles (SUV) which are similar in structure to the
LUV's except their diameters are less than 0.2 μ m.
Because of the relatively large amount of lipid in
15 the lipid bilayers of the MLV's, MLV's are considered
best for encapsulation or transportation of
lipophilic materials whereas the LUV's, because of
their large aqueous/lipid volume ratio, are
20 considered best for encapsulation of hydrophilic
molecules, particularly macromolecules. SUV's have



the advantage of small size, which allows relatively easy access to the cells of tissue, but their small volume limits delivery of hydrophilic aqueous materials to trace amounts. However, SUV's may be
5 useful in the transportation of lipophilic materials.

All of the early liposome studies used phospholipids as the lipid source for the bilayers. The reason for this choice was that phospholipids are the principal structural components of natural
10 membranes. However, there are many problems using phospholipids for liposome-type structures. First, isolated phospholipids are subject to degradation by a large variety of enzymes. Second, the most easily available phospholipids are those from natural
15 sources, e.g., egg yolk lecithin, which contain polyunsaturated acyl chains that are subject to autocatalyzed peroxidation. When peroxidation occurs, the liposome structure breaks down, causing premature release of encapsulated materials and the
20 formation of toxic peroxidation byproducts. This problem can be avoided by hydrogenation but hydrogenation is an expensive process, thereby raising the cost of the starting materials. Cost is a third problem associated with the use of
25 phospholipids on a large scale. A kilogram of egg yolk lecithin pure enough for liposome production, presently costs in excess of \$40,000. This is much too high a cost for a starting material for most applications.

30 Because of the high cost and additional problems in using phospholipids, a number of groups

have attempted to use synthetic amphiphiles in making lipid vesicles. For example, Vanlerberghe and others working for L'Oreal have used a series of synthetic polymers, primarily polyglycerol derivatives, as
5 alternatives to the phospholipids. Similarly, Kelly and a group at Sandoz, Inc. have tried aliphatic lipids.

Recently, there has been some indication, particularly from the L'Oreal group, that
10 commercially available surfactants might be used to form the lipid bilayer in liposome-like multilamellar lipid vesicles. Both surfactants and phospholipids are amphiphiles, having at least one lipophilic acyl or alkyl group attached to a hydrophilic head group.
15 The hydrophilic head groups in the surfactants which have been tried include polyoxyethylene or polyglycerol derivatives. The head groups are attached to one or more lipophilic chains by ester or ether linkages. Commercially available surfactants
20 include the BRIJ family of polyoxyethylene acyl ethers, the SPAN sorbitan alkyl esters, and the TWEEN polyoxyethylene sorbitan fatty acid esters, all available from ICI Americas, Inc. of Wilmington, Delaware.

25 No matter what starting material is used to form the MLV's, substantially all of the methods of vesicle production reported in the literature use either the original Bangham method, as described in Bangham et al., J. Mol. Biol., 13:238-252 (1965), or
30 some variation thereof. The basic approach followed starts with dissolving the lipids, together with any

other lipophilic substances including cholesterol, in an organic solvent. The organic solvent is removed by evaporation using heat or by passing a stream of an inert gas (e.g., nitrogen) over the dissolved lipid to remove the solvent. The residue is then slowly hydrated with an aqueous phase, generally containing electrolytes and any hydrophilic biologically active materials, to form large multilamellar lipid membrane structures. In some variations, different types of particulate matter or structures have used during the evaporation to assist in the formation of the lipid residue. The basis for these experiments are that by changing physical structure of the lipid residue, better vesicles may form upon hydration. Two recent review publications, Szoka and Papahdjopoulos, Ann. Rev. Biophys. Bioeng. 2:467-508 (1980), and Dousset and Douste-Blazy (in Les Liposomes, Puisieux and Delattre, Editors, Techniques et Documentation Lavoisier, Paris, pp.41-73 (1985)), summarize the methods which have been used to make MLV's.

Once the MLV's are made, it is necessary to determine the effectiveness of the process. Two measurements commonly used to determine the effectiveness of encapsulation of biological materials in liposomes or lipid vesicles are the mass of substance encapsulated per unit mass of the lipid ("encapsulated mass") and captured volume.

The captured volume is the amount of solvent trapped within the vesicles. The captured volume is defined as the concentration of the aqueous fraction

inside the vesicle divided by the concentration of lipid in the vesicle, normally given in ml/gm lipid.

Multilamellar lipid vesicles made using the classic methods have a low encapsulated mass for hydrophilic materials, normally in the order of 5-15%. In addition, the captured volume of solvent is normally in the order of 2-4 ml/g lipid. However, the encapsulated mass for lipophilic materials is much better in the multilamellar liposomes. Therefore, multilamellar liposomes made using the classical procedures are considered good for encapsulating lipophilic (hydrophobic) material but not hydrophilic.

The small unilamellar liposomes, which range in diameter from 20-50 nm, have a very low captured volume (approximately 0.5 ml/g) and also a very low encapsulated mass for hydrophilic materials (0.5-1%). However, since the lipid bilayer constitutes 50-87% of the total volume, these SUV's are excellent at transporting small quantities of lipophilic material. They also can be used to transport very small quantities of hydrophilic material to tissues where the MLV's or LUV's cannot reach.

Because of the problems in encapsulating large volumes and obtaining high encapsulated mass for hydrophilic materials, LUV's have been investigated. LUV's have large captured volumes (approximately 35 ml/gm lipid) and high encapsulated mass for hydrophilic materials (40-50%) but they are

very poor in encapsulating hydrophobic or lipophilic materials. Because of these characteristics, LUV's are best suited to encapsulation of hydrophilic materials, including macromolecules. However, there
5 are problems with the use of LUV's. Since there is only a single lipid bilayer surrounding a large aqueous center, the LUV's tend to be less stable than the other liposomes and more easily subject to degradation. Further, the low lipid/aqueous volume
10 ratio makes it difficult to use LUV's for transport of any lipophilic materials.

Although there have been some experiments reported in the literature on using synthetic surfactants rather than phospholipids as a source for
15 making multilamellar lipid vesicles, there are no reports showing any improvement in the ability to encapsulate either small or large hydrophilic molecules using these materials. In addition, there is no report of increased stability for lipid
20 vesicles made with these materials. Therefore, the literature has given no indication that liposomes manufactured with these synthetic materials will be useful to achieve the hydrophilic and macromolecule delivery objects sought in the liposome field.

25 A further problem associated with multilamellar lipid vesicles (including the small unilamellar vesicles which are normally manufactured by sonication of the multilamellar vesicles) manufactured using standard methods is that these
30 current processes are both slow and relatively inefficient in terms of material. For example, the

standard time to manufacture multilamellar lipid vesicles is in the order 2-20 hours. If SUV's are required, the sonication which breaks the multilamellar lipid structures into SUV's takes
5 additional time. This slow processing is unwieldy and expensive for any large scale use of lipid vesicles.

Accordingly, it is an object of the invention to provide a rapid and efficient process
10 for the formation of multilamellar vesicles.

It is a further object of the invention to develop multilamellar vesicles with high encapsulated mass for hydrophilic materials and high captured volume.

15 It is another object of the invention to form lipid membrane structures without the use of organic solvents or detergents.

It is still a further object of the invention to provide a method for the rapid,
20 efficient encapsulation of biologically active macromolecules into vesicles made of relatively inexpensive, readily available surfactants.

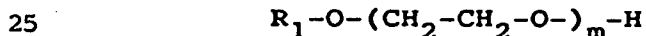
These and other objects and features of the invention will be apparent from the detailed
25 description and the claims.

Summary of the Invention

The present invention provides a method of preparing multilamellar lipid vesicles which is rapid, efficient and produces vesicles which have high encapsulated mass for hydrophilic material and high captured volumes. The invention also provides a method of encapsulating lipophilic or hydrophilic materials in high aqueous volume multilamellar vesicles with high efficiency.

In order to prepare the vesicles, a lipophilic phase is formed by blending a surfactant with a sterol and a charge producing amphiphile while maintaining the temperature of the phase above the melting point of the surfactant. The lipophilic phase is then combined with an excess of an aqueous phase under high-shear conditions and elevated temperature in order to form the multilamellar vesicles. Whereas the temperature need not be kept constant for all the formation steps, in all cases the temperature must be above the melting point of the surfactant.

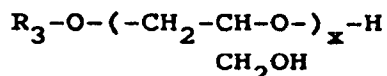
Surfactants useful in the process for forming these vesicles include polyoxyethylene acyl ethers, preferably having the structure



where R_1 is $CH_3-(CH_2)_n$, n ranges from 11 to 15, and m is 2 to 4.

Although other polyoxyethylene ethers can be used, the most preferred materials are polyoxyethylene (2) cetyl ether and polyoxyethylene (4) lauryl ether.

5 An alternative group of lipids which are also useful in the invention, are the polyglycerol acyl ethers, preferably having the structure



where R_3 is $\text{CH}_3\text{-(CH}_2)_y$, y ranges from 11 to 15, and x ranges from 1 to 3.

10 The purpose of the sterol in the vesicles is to buffer the thermotropic phase transition of the membrane layer with insures optimal size and provides high stability, including stability near the transition temperature of the lipid. The most
15 preferred sterol is cholesterol but any sterol having similar properties will provide similar results.

Vesicles made without charge producing materials lack the capacity for high volume uptake and efficient incorporation of hydrophilic molecular
20 and macromolecules; they also have the tendency to aggregate or clump, making them unusable for most applications. Because of this, a charge producing material is used in the method of the invention to provide a net charge, either positive or negative, to
25 the formed vesicle. The preferred negative charge producing materials are selected from a group consisting of dicetyl phosphate, cetyl sulphate,

certain long chain fatty acids, retinoic acid, phosphatidic acid, phosphatidyl serine, and mixtures thereof.

5 In order to provide a net positive charge to the vesicles, long chain amines, long chain pyridinium compounds (e.g., cetyl pyridinium chloride), quaternary ammonium compounds or mixtures thereof can be used. A preferred material for causing a positive charge is hexadecyl
10 trimethylammonium bromide, a potent disinfectant. The use of this disinfectant as a positive charge producing material within the vesicles provides a secondary advantage as the vesicles deteriorate; they act as a sustained release germicide carriers.

15 The vesicles may also include targeting molecules, either hydrophilic or amphiphilic, which can be used to direct the vesicles to particular targets in order to allow release of the material encapsulated in the vesicle at a specified biological
20 location. If hydrophilic targeting molecules are used, they can be coupled directly or via a spacer to an OH residue of the polyoxyethylene or polyglycerol portion of the surfactant, or they can be coupled, using state of the art procedures, to molecules such
25 as palmitic acid or phosphatidylethanolamine. If spacers are used, the targeting molecules can be interdigitating with the hydrophilic core of the bilayer membrane via the acyl chains of these compounds. Preferred hydrophilic targeting molecules
30 include monoclonal antibodies, lectins, and peptide hormones.

In addition to hydrophilic targeting molecules, it is also possible to use amphiphilic targeting molecules. Amphiphilic targeting molecules are normally not chemically coupled to the surfactant molecules but rather interact with the lipophilic or hydrophobic portions of the molecules constituting the bilayer lamellae of the lipid vesicles. Preferred amphiphilic targeting molecules are neutral glycolipids, galactocerebrosides, (e.g., for hepatic galactosyl receptors), or charged glycolipids, such as gangliosides.

Vesicles made using the methods of the present invention can be used in diagnostic testing, e.g., agglutination testing of immunological systems. The vesicles can also be used as markers or labels for visualization, e.g., for radiography.

In another aspect, the invention provides a method of encapsulating hydrophilic or lipophilic materials. In order to encapsulate lipophilic materials within the vesicle, the lipophilic materials are blended into the lipophilic phase formed of the surfactant, a sterol and a charge producing material at a temperature above the melting temperature of the surfactant. The formation of the vesicle is otherwise carried out as previously described.

In order to encapsulate a hydrophilic material, the lipophilic phase is made as previously described and the hydrophilic material to be encapsulated is added to the aqueous phase.

Hydrophilic materials which can be encapsulated include macromolecules, viruses, immunological adjuvants such as muramyl dipeptide, peptide hormones such as insulin, glucagon, and pituitary hormones, 5 growth factors such as angiogenic, epithelial and epidermal growth factors, lymphokines such as interleukin-2 and interferon, blood proteins such as hemoglobin, water-soluble plant hormones and pesticides, radionucleotides, and contrast dyes for 10 radiological diagnosis. Examples of lipophilic materials which can be encapsulated include steroid hormones, organic pesticides, fungicides, insect repellants, and lipophilic vitamins and derivatives. A more complete listing of the types of materials 15 that could be used in lipid vesicles is included in an article by Gregoriadis, New Engl. J. Med. 295:704-711 (1976).

The following description and examples more fully illustrate the invention.

20 Description

The present invention features a process of making a new type of multilamellar lipid vesicle with large aqueous volume using surfactants as the lipid source in a rigid production method, a method of 25 encapsulating hydrophilic or lipophilic materials within this type of multilamellar lipid vesicle, and the high aqueous volume multilamellar lipid vesicles themselves. Based on encapsulated mass and captured volume, the multilamellar lipid vesicles of the 30 invention appear better suited to the encapsulation

and delivery of hydrophilic materials, including macromolecules, than multilamellar lipid vesicles known in the art. Further, by using the most preferred materials to form the multilamellar lipid vesicles, these vesicles appear to tolerate a broader range of pH than classic liposomes or other known multilamellar lipid vesicles and are not as susceptible to attack by oxidative systems, e.g., peroxidases and superoxide-generating systems of phagocytes. The multilamellar lipid vesicles are also much cheaper to make because of a lower cost of the starting materials.

In broad terms, the multilamellar lipid vesicles of the present invention are made by raising the temperature of the lipid structural materials, which may be polyoxyethylene acyl ethers or polyglycerol acyl ethers, to a temperature above their melting point so that they are liquid. A sterol, preferably cholesterol, together with a charge producing material and any lipophilic materials to be encapsulated is blended into the liquid surfactant to form a lipophilic phase. This lipophilic phase is then forced into an excess of an aqueous phase, also at a temperature above the melting point of the surfactant, using a high shear device. If any hydrophilic materials are to be encapsulated within the multilamellar lipid vesicles, they are included in the aqueous phase. Since the polyoxyethylene acyl ethers useful in the invention have low melting points, bioactive hydrophilic materials which are temperature-sensitive can still be encapsulated without damage. This permits the

present method to be used for a broad range of materials.

Anionic or cationic amphiphiles are incorporated into the surfactant to yield a net negative or positive charge. The incorporation of a charge-bearing material into the lipid structure stabilizes the lipid structure and provides rapid dispersion. If such a charge is not used, any vesicles formed will aggregate unless they are kept at very low concentrations. The charged material is also required for a large aqueous volume to be encapsulated. The amount of charged amphiphile does not have to be large, 0.5 moles percent - 5 moles percent (based on the concentration of the surfactant) is sufficient to provide proper charge to the vesicles.

Cholesterol, or another sterol with similar chemical properties, is incorporated into the lipid structure of the multilamellar vesicles in order to provide better stability and buffer the thermotropic phase transition of the membrane layer, e.g., providing stability of the membrane structure at temperature near the transition temperature of the lipid. The cholesterol also permits optimum size of the finished vesicle. The preferred surfactant/cholesterol molar ratio ranges from about 3-20, and depends to some extent on whether cholesterol competes with any lipophilic material to be encapsulated.

Although the polyoxyethylene and polyglycerol surfactants described herein are the best presently known for carrying out the method of the invention, it is possible that phospholipids or
5 other surfactants could be used to form vesicles by this method. However, many of these phospholipids and other surfactants have such high melting temperature that it would be impractical to use these for encapsulating biologically active materials which
10 are temperature sensitive. Further, if more unsaturated lipids are used, they are more susceptible to oxidative breakdown.

Once the lipophilic phase is formed, it is necessary to hydrate it using a high shear
15 technique. There are a large variety of devices available on the market which can provide this high shear. Devices which could be used include a microfluidizer such as is made by Biotechnology Development Corporation, a "French"-type press, or
20 some other device which provides a high enough shear force and the ability to handle heated, semiviscous lipids. If a very high shear device is used, it may be possible to microemulsify powdered lipids, under pressure, at a temperature below their normal melting
25 points and still form the multilamellar lipid vesicles of the present invention.

Once the multilamellar lipid vesicles are formed, the size can be changed or the structure modified by sonication or mechanical shear. Devices
30 for carrying this out, as well as the general

procedures, are known to those skilled in the art and are commonly used in the liposome field.

If the multilamellar lipid vesicles of the present invention are used as a drug-delivery system, there is no particular limitation on how they can be used. For example, the vesicles may be dispersed directly in suspension, in aerosol form, topically, or in a gel. If used for agglutination testing or some other type of marker use, lipophilic dyes which are taken up directly into the lipid layers may be used.

In addition to use as a drug or macromolecule delivery system, the multilamellar lipid vesicles of the invention have substantial other uses. For example, the vesicles can be used as an adjuvant in order to improve the immunological response of injected material. In addition, the high aqueous volume allows the use of the multilamellar lipid vesicles of the invention as moisturizers or skin creams with advantageous results. The high captured volume/lipid ratio is such that more moisture is provided to the skin using the vesicles of the invention than is available from conventional skin care creams.

The invention will be more apparent from the following, non-limiting Examples.

Example 1.

The multilamellar lipid vesicles of this Example were made using one of the most preferred materials, polyoxyethylene (2) cetyl ether. Although
5 syringes were used as described to provide the high shear in this and the following Examples, any high shear device could have been used.

TABLE 1

	Polyoxyethylene (2) cetyl ether	0.696 gm
10	Cholesterol	0.073 gm
	Dicetyl phosphate	0.055 gm
	5 mM phosphate, 150 mM NaCl, pH 7.4	10.0 ml

Table 1 lists the materials and proportions used in preparing the multilamellar lipid vesicles
15 for this Example. The polyoxyethylene (2) cetyl ether, cholesterol and dicetyl phosphate were placed in a 5 ml syringe and heated to 40°C., a temperature above the melting point of the lipid. The dicetyl phosphate provided a net negative charge
20 to the final membrane structure. The lipophilic phase which resulted after the heating and blending of the lipophilic components was forcibly injected, via a three-way stopcock, into an aqueous phase consisting of 10 ml of 5 mM phosphate buffer
25 containing 150 mM NaCl, pH 7.4. The phosphate buffer, which was contained in a 25 ml syringe, was also at 40°C. The process of injection of the

lipophilic phase into the aqueous phase took less than five seconds. The resulting mixture was then forced into a second 25 ml syringe at a linear flow rate of $8-12 \times 10^2$ cm/sec through an orifice about 1 mm in diameter. The mixture was driven continuously back and forth between the two 25 ml syringes for approximately 2 minutes, providing the liquid shear necessary to make the high volume lipid vesicles. A milky suspension containing the multilamellar lipid vesicles resulted. The multilamellar lipid vesicles were separated by centrifugation at 10,000 rpm for 15 minutes in a Beckman Instrumental Co. J-21 centrifuge, forming a low density phase on top of the aqueous solution.

The multilamellar lipid vesicles formed would not pass through a $0.8 \mu\text{m}$ filter. Upon sonication for 6 minutes in a Branson sonicator, the lipid membrane structures attained the size of normal multilamellar vesicles, passing through a $0.45 \mu\text{m}$ filter. Upon sonification for an additional 6 minutes, the structures were reduced enough in size to pass through a $0.2 \mu\text{m}$ filter.

Example 2.

In this Example, the identical procedure was used as in Example 1 except the dicetyl phosphate, which provided a negative charge in Example 1, was replaced by cetyl trimethylammonium. The exact proportions used in this Example are shown in Table 2.

TABLE 2

	Polyoxyethylene (2) cetyl ether	0.696 gm
	Cholesterol	0.073 gm
	Cetyl trimethylammonium	0.036 gm
5	5 mM phosphate, 150 mM NaCl, pH 7.4	10.0 ml

The positively charged multilamellar vesicles produced again could not pass through a 0.8 μ m filter but upon sonification for 6 minutes, they passed freely through a 0.45 μ m filter. Upon
10 further sonification for an additional 6 minutes, the lipid membrane structures again passed freely through a 0.2 μ m filter.

Example 3.

In this Example, a larger scale test was
15 made using the same materials as Example 1. Three grams of lipid were employed. The molar proportions of the material used, as well as the volume of aqueous phase, are disclosed in Table 3.

TABLE 3

20	Polyoxyethylene (2) cetyl ether	33	mM
	Cholesterol	11	mM
	Dicetyl phosphate	1.5	mM
	5 mM phosphate, 150 mM NaCl, pH 7.4	50	ml

The polyoxyethylene (2) cetyl ether, the cholesterol, and the dicetyl phosphate, a total of 3 gm of lipid, were placed in a 25 ml syringe and heated to 40°C. The mixture was then forcibly
5 injected, via a three-way stopcock, into 50 ml of the phosphate buffer, also at 40°C., contained in a 60 ml syringe. This process took less than 10 seconds. The resulting mixtures were then forced into a second 60 ml syringe at a flow rate of $8-12 \times 10^2$ cm/sec
10 through an orifice about 1 mm in diameter. The resulting mixture was driven continuously back and forth between the two 60 ml syringes for about two minutes, yielding a cream. Upon centrifugation at 10,000 rpm for 15 minutes, the lipid membrane
15 structure was separated as a layer atop the nonincorporated aqueous phase. The captured aqueous volume in different experiments was 7-20.8 ml/g lipid, an amount much greater than the 2-4 ml/g lipid generally observed for multilamellar lipid membrane
20 structures. A 1/100 dilution of the vesicles was found to be stable against aggregation for thirty days at ambient temperature.

Example 4.

In this Example, substantially the same
25 methods were used as in Example 3 except polyoxyethylene (4) lauryl ether was used in place of the polyoxyethylene (2) cetyl ether. Since the lauryl ether is a liquid at ambient temperature, no heating was required. Three grams of total lipid was
30 used, with the proportions given in Table 4.

TABLE 4

	Polyoxyethylene (4) lauryl ether	33	mM
	Cholesterol	11	mM
	Dicetyl phosphate	1.5	mM
5	5 mM phosphate, 150 mM NaCl, pH 7.4	50	ml

After formation of the multilamellar lipid vesicles and separation by centrifugation, the captured volume was measured and found to be 8 ml/g lipid. This is entirely surprising since the
10 multilamellar lipid vesicles formed in this experiment passed freely through a 0.2 μ m filter without sonification. Because of this small size, the lauryl vesicles may have similar access to organs that SUV's have while still allowing high captured
15 volume and encapsulation efficiency.

Example 5.

In this Example, a macromolecule, specifically hemoglobin, was used to show encapsulation efficiency for the multilamellar lipid
20 vesicles of the invention. The polyoxyethylene (2) cetyl ether was used to prepare the lipid membrane structures. Table 5 lists the concentrations.

1289420

-22-

TABLE 5

	Polyoxyethylene (2) cetyl ether	3.1	gm
	Cholesterol	0.7	gm
	Dicetyl phosphate	0.13	gm
5	Red cell hemolysate (10 mg Hb/ml)	50	ml

The red cell hemolysate was formed by lysing fresh, washed human erythrocytes in hypotonic phosphate buffer to give a hemoglobin concentration of 10 mg/ml. The lipid, cholesterol and dicetyl phosphate were placed in a 10 ml syringe and heated to 40°C. The mixture was then forcibly ejected, via a three-way stopcock, into 50 ml of the red cell hemolysate contained in a 60 ml syringe. This injection took less than 5 seconds. The resulting mixture was then forced into a second 60 ml syringe at a flow rate of $8-12 \times 10^2$ cm/sec through an orifice of about 1 mm. The resulting mixture was driven continuously back and forth between the two syringes for approximately 2 minutes, yielding a dark pink cream.

Seven ml of the resulting cream was mixed with 3 ml of a Ficoll-Paque density barrier (Pharmacia) and centrifuged at 10,000 rpm for 15 minutes. Any unincorporated hemoglobin stays in the Ficoll-Paque density barrier whereas hemoglobin associated with the lipid vesicles will float with the lipophilic phase to the top of the aqueous phase. The lipophilic, vesicle-containing phase was

pink colored and separated from the top of the density barrier. One ml aliquots of the two fractions (the lipid phase and the density barrier phase) were dissolved in 4 ml of Soluene (0.5 N quaternary ammonium hydroxide in toluene, made by Packard) and the hemoglobin content was determined by measuring the absorbance of the Soret band (420 nm). The Ficoll-Paque had a 0.42 O.D. while the lipid membrane structures had a 1.46 O.D., showing that about 22 mg of hemoglobin per gram lipid was associated with the lipid membrane structures. The corresponding aqueous volume uptake was approximately 8 ml/g.

Gassing with moist nitrogen caused the characteristic spectral change in the hemoglobin associated with the lipid membrane structures, showing a transformation from oxyhemoglobin to deoxyhemoglobin. After reexposure to ambient oxygen, the spectral change occurred, showing a transformation back to oxyhemoglobin. This illustrates that the hemoglobin is unharmed by the encapsulation process.

The hemoglobin containing structures were kept in buffer for 11 days at 40°C. then repurified on a Ficoll-Paque density barrier. Seventy percent of the hemoglobin that was encapsulated was still found to be present in the lipid phase. The hemoglobin-containing lipid membrane structures still illustrated the deoxygenation-reoxygenation reaction. A similar experiment at 17 days showed that 62% of the hemoglobin initially incorporated was

still retained and still exhibited normal deoxygenation-reoxygenation.

A similar experiment was run using 30 mg hemoglobin/ml, a three-fold increase in concentration. An expected increase in hemoglobin encapsulation, 58 mg/g lipid, was observed.

Example 6.

In this Example, polyoxyethylene (10) cetyl ether was compared with polyoxyethylene (2) cetyl ether in order to determine encapsulated mass and captured volume. The proportions of the materials used were identical to those shown in Table 1. Table 6 gives the results of this experiment.

TABLE 6

15	Surfactant	Volume taken up (ml/g lipid)	Hemoglobin taken up mg/g lipid
	Polyoxyethylene (2) cetyl ether	7-9	20-60
20	Polyoxyethylene (10) cetyl ether	2-3	<3

For the polyoxyethylene (2) cetyl ether, 7-9 ml solvent/g lipid was taken up into the aqueous volume and the encapsulated mass for the hemoglobin was 20-60 mg/g lipid. In contrast, using the polyoxyethylene (10) cetyl ether only 2-3 ml

solvent/g lipid was taken up and the encapsulated mass was less than 3 mg/g lipid. The values for the polyoxyethylene (10) cetyl ether are substantially the same as those shown in the literature using classic encapsulation methods, and phospholipids, using phospholipids and classic encapsulation methods for the formation of MLV. This shows that the method of the invention works for a variety of materials; however, the polyoxyethylene (2) cetyl ether yields a clear advantage.

Example 7.

In this Example, a lipophilic molecule, specifically retinoic acid, used to demonstrate the capacity of the multilamellar vesicles of this invention to encapsulate lipophilic molecules. The polyoxyethylene (2) cetyl ether was used as the lipid structural material of the vesicles. The retinoic acid is incorporated into the lipophilic phase of the lipid membrane structures. Two and a half grams total lipid was employed in the proportions given in Table 7 and the method used was that of Example 3.

TABLE 7

	Polyoxyethylene (2) cetyl ether	33	mM
	Cholesterol	6	mM
25	Dicetyl phosphate	1.5	mM
	Retinoic Acid	0.67	mM
	5 mM phosphate, 150 mM NaCl, pH 7.4	40	ml

In accordance with the method of this invention, the polyoxyethylene (2) cetyl ether, cholesterol, dicetyl phosphate and retinoic acid were blended at 40°C. in a 10 ml syringe and the mixture
5 was then forcibly injected into 40 ml 5mM phosphate, 150 mM NaCl, pH 7.4, likewise at 40°C., in a 60 ml syringe. The mixture was then subjected to high fluid shear by two minutes of mixing through a 1 mm orifice into another 60 ml syringe, yielding a yellow
10 cream.

Upon centrifugation at 15,000 rpm for 15 minutes, the lipid vesicles separated as a yellow layer atop the nonincorporated aqueous phase. The isolated lipid vesicles could be diluted without
15 further volume uptake to form a stable, homogeneous suspension. The measured incorporation of aqueous phase into the lipid membrane structures was 18 ml/g. This very high value under the conditions employed may be due to the added net negative charge
20 contributed by the retinoic acid. The encapsulation of retinoic acid was 8 mg/g lipid (>99%).

Example 8.

In this Example, retinoic acid was used to replace dicetyl phosphate in providing the negative
25 charge for lipid vesicles prepared with polyoxyethylene (2) cetyl and cholesterol. Two and a half grams of a lipid mixture with the molar proportions in Table 8 was employed. The method used was identical with that of Example 3.

TABLE 8

	Polyoxyethylene (2) cetyl ether	33	mM
	Cholesterol	6	mM
	Retinoic acid	1.5	mM
5	5 mM phosphate, 150 mM NaCl, pH 7.4	40	ml

After formation of the multilamellar vesicles and separation by centrifugation, the aqueous volume taken up was measured and found to be 12 ml/g lipid. The retinoic acid encapsulated was
10 17.5 mg/g.

Example 9.

This Example demonstrates the capacity of the lipid vesicles formed by the method of this invention from polyoxyethylene (2) cetyl ether were
15 to incorporate a different lipophilic material, the insect repellant N,N-diethyl meta-toluamide. Two and a half gram of lipid was used in the proportions given in Table 9. The method used was the same as
Example 7 with the N,N-diethyl meta-toluamide
20 replacing the retinoic acid.

TABLE 9

	Polyoxyethylene (2) cetyl ether	33	mM
	N,N-diethyl meta-toluamide	11	mM
	Cholesterol	5	mM
5	Dicetyl phosphate	1.5	mM
	5 mM phosphate, 150 mM NaCl, pH 7.4	40	ml

Upon centrifugation at 15,000 rpm for 15 minutes, the lipid membrane structures separated as a white layer atop the nonincorporated aqueous phase.

10 This could readily be redispersed and diluted into a uniform suspension without separation of a low-density phase of N,N-diethyl meta-toluamide. The volume uptake was 10 ml/g lipid and >99% of the N,N-diethyl meta-toluamide was retained by the lipid

15 membrane vesicle. Separate experiments showed that if cholesterol is eliminated from the system, the liposomes quickly lost the N,N-diethyl meta-toluamide.

Example 10.

This Example demonstrates the capacity of

20 the lipid vesicles formed by the method of this invention to encapsulate supramacromolecular structures, specifically avian encephalitis (AE) virus, a 17 nm virion. The proportions and method used are identical to those of Example 5 except the

25 red blood lysate was replaced by a solution of the AE virus. The results are shown in Table 10.

1289420

-29-

TABLE 10

SERUM DILUTION		1:00	1:2	1:4	1:8	1:16	1:32
SAMPLE							
5	AE VIRUS standard and used for incorporation	1.47	0.75	0.48	0.24	0.21	0.17
	AQUEOUS RESIDUE	0.08	0.08	0.10	0.08	0.12	0.99
CONTROL AVERAGE = 0.077							
10	STANDARD-CONTROL	1.39	0.67	0.40	0.16	0.13	0.09
	RESIDUE-CONTROL	0.00	0.00	0.02	0.00	0.04	0.02

As is evident from the results of Table 10,
at least 75% of AE is taken up into the multilamellar
vesicles of this invention, indicating their
15 potential usefulness in the transportation of viruses
and plasmids.

Example 11.

In this Example, the percent uptake of an
aqueous based solution was determined for
20 multilamellar vesicles of the invention. The
vesicles were made as disclosed in Example 1 except

2.5 grams of lipid was used to form the lipophilic phase while different amounts of a 0.25 N sorbitol solution was offered as an aqueous phase. The lipid was then separated by density gradient centrifugation and the volumes were measured. Table 11 illustrates the captured volume in ml/g of lipid.

TABLE 11

	Offered volume	Volume taken up	Volume taken up/g	% uptake
10	10 ml	10 ml	4	100
	20 ml	20 ml	8	100
	30 ml	30 ml	12	100
	40 ml	40 ml	16	100
	50 ml	48 ml	19.2	96
15	60 ml	52 ml	20.8	87

As is evident from the results shown in Table 11, the multilamellar vesicles of the present invention have much greater captured volume than conventional multilamellar vesicles.

20 What is claimed is:

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method of preparing high aqueous volume multilamellar lipid vesicles comprising the steps of:

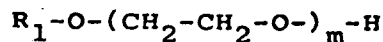
A. providing a solventless lipophilic phase by blending a polyoxyethylene fatty ether surfactant with a sterol and a charge producing amphiphile while maintaining the temperature of said lipophilic phase above the melting point of said surfactant;

B. providing an aqueous phase formed of an aqueous solvent and any aqueous soluble materials to be encapsulated; and

C. combining said non-aqueous lipophilic phase with a substantial excess of said aqueous phase in a single step under shear conditions while maintaining the temperature above the melting point of said surfactant;

whereby said high aqueous volume multilamellar lipid vesicles are formed in less than two minutes without forming a separable hydrated lamellar phase.

2. The method of claim 1 wherein said surfactant comprises a polyoxyethylene fatty ether having the structure



1289420

where R_1 is $\text{CH}_3-(\text{CH}_2)_n$, n ranges from 11 to 15, and m is 2 to 4.

3. The method of claim 2 wherein said sterol comprises cholesterol or a derivative thereof.

4. The method of claim 3 wherein said charge producing amphiphile is a negative charge producing material selected from a group consisting of dicetyl phosphate, cetyl sulphate, long chain fatty acids, stearic acid, phosphatidic acid, phosphatidyl serine, and mixtures thereof.

5. The method of claim 3 wherein said charge producing amphiphile is a positive producing material selected from a group consisting of long chain amines, long chain pyridinium compounds, quaternary ammonium compounds, and mixtures thereof.

6. The method of claim 3 further comprising coupling a hydrophilic targeting molecule selected from a group consisting of monoclonal antibodies, lectins and peptide hormones to said surfactant, said hydrophilic targeting molecule being coupled directly to an OH residue of the polyoxyethylene portion of said surfactant.

7. The method of claim 3 further comprising coupling a hydrophilic targeting molecule selected from a group consisting of monoclonal antibodies, lectins and peptide hormones to said surfactant, said hydrophilic targeting molecule being coupled through a spacer molecule to an OH residue of the polyoxyethylene portion of said surfactant.

8. The method of claim 3 further comprising coupling a hydrophilic targeting molecule selected from a group consisting of monoclonal antibodies, lectins and peptide hormones to said surfactant, said hydrophilic targeting molecule being coupled directly to an acyl chain interdigitating with those of said surfactant.

9. The method of claim 3 further comprising coupling a hydrophilic targeting molecule selected from a group consisting of monoclonal antibodies, lectins and peptide hormones to said surfactant, said hydrophilic targeting molecule being coupled through a spacer molecule to an acyl chain interdigitating with those of said surfactant.

10. The method of claim 3 wherein said polyoxyethylene fatty ether comprises polyoxyethylene (2) cetyl ether.

11. The method of claim 3 wherein said polyoxyethylene fatty ether comprises polyoxyethylene (4) lauryl ether.

12. The method of claim 3 wherein said charge producing molecule comprises retinoic acid.

13. A method for encapsulating an amphiphilic material within a multilamellar lipid vesicle consisting essentially of the steps of:

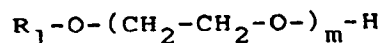
A. providing a solventless non-aqueous lipophilic phase by blending a polyoxyethylene fatty ether surfactant with a sterol and a charge producing amphiphile while maintaining the temperature of said lipophilic phase above the melting point of said surfactant, and blending said amphiphilic material to be encapsulated into said lipophilic phase;

B. providing an aqueous phase formed of an aqueous solvent and any aqueous soluble materials to be encapsulated; and

C. combining said non-aqueous lipophilic phase with a substantial excess of said aqueous phase in a single step under shear conditions while maintaining the temperature above the melting point of said surfactant;

whereby said multilamellar lipid vesicles are formed and said amphiphilic material is encapsulated in less than two minutes without forming a separable hydrated lamellar phase.

14. The method of claim 13 wherein said surfactant comprises a polyoxyethylene fatty ether having the structure



where R_1 is $CH_3-(CH_2)_n$, n ranges from 11 to 15, and m is 2 to 4.

15. The method of claim 14 wherein said sterol comprises cholesterol or a derivative thereof.

16. The method of claim 15 wherein said charge producing amphiphile is a negative charge producing material selected from a group consisting of dicetyl phosphate, cetyl sulphate, long chain fatty acids, retinoic acid, phosphatidic acid, phosphatidyl serine, and mixtures thereof.

17. The method of claim 15 wherein said charge producing amphiphile is a positive charge producing material selected from a group consisting of long chain amines, long chain pyridinium compounds, quaternary ammonium compounds, and mixtures thereof.

18. The method of claim 15 wherein said polyoxyethylene fatty ether comprises polyoxyethylene (2) cetyl ether.

19. The method of claim 15 wherein said polyoxyethylene fatty ether comprises polyoxyethylene (4) lauryl ether.

20. A method of encapsulating a hydrophilic material within a high aqueous volume multilamellar lipid vesicle consisting essentially of the steps of:

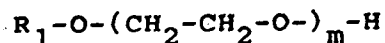
A. providing a solventless non-aqueous lipophilic phase by blending a polyoxyethylene fatty ether surfactant with a sterol and a charge producing amphiphile while maintaining the temperature of said lipophilic phase above the melting point of said surfactant;

B. providing an aqueous phase by blending said hydrophilic material to be encapsulated into an aqueous solvent; and

C. combining said non-aqueous lipophilic phase with a substantial excess of said aqueous phase in a single step under shear conditions while maintaining the temperature above the melting point of said surfactant,

whereby said high aqueous volume multilamellar lipid vesicles are formed and said hydrophilic material is encapsulated in less than two minutes without forming a separable hydrated lamellar phase.

21. The method of claim 20 wherein said surfactant comprises a polyoxyethylene fatty ether having the structure



1289420

where R_1 is $\text{CH}_3-(\text{CH}_2)_n$, n ranges from 11 to 15, and m is 2 to 4.

22. The method of claim 21 wherein said sterol comprises cholesterol or a derivative thereof.

23. The method of claim 22 wherein said charge producing amphiphile is a negative charge producing material selected from a group consisting of dicetyl phosphate, cetyl sulphate, long chain fatty acids, retinoic acid, phosphatidic acid, phosphatidyl serine, and mixtures thereof.

24. The method of claim 22 wherein said charge producing amphiphile is a positive charge producing material selected from a group consisting of long chain amines, long chain pyridinium compounds, quaternary ammonium compounds, and mixtures thereof.

25. The method of claim 22 wherein said polyoxyethylene fatty ether comprises polyoxyethylene (2) cetyl ether.

26. The method of claim 22 wherein said polyoxyethylene fatty ether comprises polyoxyethylene (4) lauryl ether.

1289420

27. The method of claim 20 wherein said hydrophilic material comprises a macromolecule.

28. The method of claim 27 wherein said hydrophilic material comprises hemoglobin.

29. The method of claim 20 wherein said hydrophilic material comprises a peptide hormone.

30. The method of claim 20 wherein said hydrophilic material comprises a growth factor.

31. The method of claim 20 wherein said hydrophilic material comprises a lymphokine.

32. The method of claim 20 wherein said hydrophilic material comprises interleukin.

33. The method of claim 20 wherein said hydrophilic material comprises interferon.

34. The method of claim 20 wherein said hydrophilic material comprises a virus.



SUBSTITUTE

REMPLACEMENT

SECTION is not Present

Cette Section est Absente



Ottawa Hull K1A 0L9

(21)	(A1)	2,160,775
(86)		1994/04/25
(43)		1994/11/24

(51) Int.Cl. ⁶ A61K 9/70

(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) **Transdermal Therapeutic System Containing Estradiol**

(72) Meconi, Reinhold - Germany (Federal Republic of) ;
Seibertz, Frank - Germany (Federal Republic of) ;
Horstmann, Michael - Germany (Federal Republic of) ;
Lichtenberger, Rainer - Germany (Federal Republic of) ;

(71) LTS Lohmann Therapie-Systeme GmbH & Co. KG - Germany
(Federal Republic of) ;
Merck Patent GmbH - Germany (Federal Republic of) ;

(30) (DE) P 43 14 970.7 1993/05/06
(DE) P 43 36 557.4 1993/10/27

(57) 23 Claims

Notice: This application is as filed and may therefore contain an incomplete specification.

2160775

ABSTRACT

The invention concerns a transdermal therapeutic system designed for the controlled release of estradiol or any of its pharmaceutically acceptable derivatives, alone or in combination with gestagens, the system comprising a back film, an active-substance reservoir which is joined to the back film and is produced using pressure sensitive adhesives, and a detachable protective film. The system is characterized in that the pressure sensitive adhesive contains esters of colophonium.

Transdermal Therapeutic System Containing Estradiol

S P E C I F I C A T I O N

The present invention relates to a transdermal therapeutic system for the controlled release of estradiol or its pharmaceutically acceptable derivatives alone or combined with gestagens, such as levonorgestrel, to human or animal skin. The present invention further relates to the use and to a process for the production of this system.

In the therapy of various diseases transdermal therapeutic systems (TTS) have been introduced on the market. Also, transdermal therapeutic systems containing the estrogenic active substance 17- β -estradiol used as therapeutic agent for climacteric complaints and - for some time now - against osteoporosis are commercially available and show good therapeutic results.

Levonorgestrel is a synthetic gestagen derivative which has mainly been used in contraceptives in combination with orally effective estrogens. In such preparations gestagens, consequently including levonorgestrel, have the function to cause a "physiologic" abstraction hemorrhage which is as short and rapid as possible by means of an adequate trophic premedication of the uterus. There are also hints that the gestagen addition has a protective effect against the risk of endometrial tumors.

For this reason, it is appropriate to use a cyclic treatment also for the indication of postmenopausal complaints, i.e., to make use of a temporary fixed drug combination consisting of estrogens (e.g., estradiol) and gestagens (e.g., levonorgestrel). A combination of the two active substances in a common, monolithic transdermal therapeutic system which would have to be applied only once a

day or even once to twice a week is particularly interesting. Owing to its high efficiency and permeativity through the skin levonorgestrel is excellently suitable for such a system.

Experimental systems for the transdermal delivery of levonorgestrel are described in literature (Friend et. al., J. Controlled Release 7, 243-250 (1988)). However, according to this estimation, permeation improvers (enhancers), e.g., alkyl esters of short-chain fatty acids, are required for the successful transdermal therapy with sufficiently small system surfaces (Friend et. al., J. Controlled Release 9, p. 33-40 (1989)).

Numerous devices for the transdermal application of estrogens and gestagens have been disclosed. Nakagawa et al. (EP-A 0 483 370) obtained a matrix-type transdermal therapeutic system for estradiol alone by using styrene-isoprene block copolymer, moisture-absorbing polymer domains, and the enhancer (and antipruritic agent) crotonal. Another conception is the simultaneous application of estradiol and an enhancer (ethanol) in a membrane-controlled reservoir system (Campbell et al., US-PS 4 379 454); this can also be used in a combined administration form comprising the gestagen norethisterone acetate (Frankhauser and Schenkel, DE 3 810 896).

However, transdermal therapeutic systems for the release of estradiol and/or gestagens have the disadvantage that they either contain ethanol or that they exhibit the potential danger of the active substance being recrystallized in the course of time.

It is known from DE-OS 32 05 258 and EP 0 285 563 to administer estradiol and ethanol simultaneously in a patch formulation. However, the production of this patch is very expensive, and the

wearing comfort after application is low because of missing flexibility.

EP O 285 563 describes a transdermal therapeutic system for the combined application of estrogens and gestagens. The reservoir has the active substance formulation, optionally a membrane, and ethanol as percutaneous absorption improving agent. Since the release of the active substance is mainly controlled by the membrane, this transdermal therapeutic system is completely different from the active-substance-containing patch according to the present invention. In the patch described in said publication, the adhesive has the mere function of fastening the patch to the skin. The fact that it can contribute to the control of the active substance release is not its main function but merely a - probably even undesired - side effect. It is a so-called "pouch patch" since the active substance preparation is present in a pouch consisting of an impermeable backing layer and a membrane having an adhesive layer. As a consequence of its complicated structure, the production of this patch is very expensive since the individual components have to be produced separately and then joined in an additional step to form a patch.

EP O 275 716 describes a two-layer transdermal therapeutic system - in contrast to the single-layer system according to the present invention - for the simultaneous administration of one or several estrogens which are dissolved or microdispersed in the polymeric layer. In addition to the active substances, the pressure sensitive adhesive layer comprises substances improving the transdermal absorption. Polymeric and pressure sensitive adhesive layer may consist of polyacrylates, silicones, or polyisobutylenes.

EP O 072 251 describes a flexible, liquid-absorbing medicinal bandage. The substrate which is attached to the flexible backing

layer consists of a hydrophilic matrix based on hydrophilic high-molecular polysaccharides and/or polyacrylic acid, polyacrylamide, ethylene-vinyl acetate-copolymers, and other polymers as well as of a liquid phase based on a solution or emulsion of carbohydrate, proteins, multivalent alcohols, and different active substances, amongst others hormones. The main feature of this invention is the moisture-absorbing adhesive.

EP 0 328 806 describes a transdermal therapeutic system without membrane; its matrix consists of a polyacrylate adhesive, a solvent, a penetration enhancer, and estrogens, the derivatives and combinations thereof.

WO 87/07 138 describes an estradiol patch based on a backing layer, an active-substance-containing matrix and a pressure sensitive adhesive covered with a removable protective layer. The matrix and pressure sensitive adhesive are manufactured in technologically very expensive operations by homogenizing, degassing, coating, drying, and separating. According to an embodiment, the backing layer has to be coated with a pressure sensitive adhesive, resulting in an additional operation. The individual parts are joined in a separate step. For this reason, the production of this patch is very expensive and complicated.

US-PS 4 624 665 describes systems comprising the active substance in microencapsulated form within the reservoir. The reservoir is embedded between the backing layer and a membrane. The outer edge of the system is provided with a pressure sensitive adhesive. The structure and the production of this system are very complicated since the active substance has to be microencapsulated and homogeneously distributed in a liquid phase which is then embedded between backing layer and membrane in additional

process steps. In addition, this system must then be provided with an adhesive edge and covered with a protective layer.

Additionally, EP O 186 019 describes active substance patches wherein water-swellaable polymers are added to a rubber/adhesive-resin-mass and from which estradiol can be released. However, it turned out that the release of estradiol from these active substance patches is too low and does not meet the therapeutic requirements.

DE-OS 20 06 969 describes a patch or pressure sensitive adhesive dressing exhibiting system action; it contains contraceptive substances which are incorporated in the adhesive component or in the adhesive film. This publication discloses that the adhesive may be an acrylate.

DE-OS 39 33 460 describes an estrogen-containing active substance patch based on homo and/or copolymers with at least one derivative of the acrylic acid or with methacrylic acid in combination with water-swellaable substances.

However, it turned out that pressure sensitive adhesive transdermal therapeutic matrix systems which comprise the active substance in a partially or completely dissolved form involve the potential risk that the active substance recrystallizes in the course of time. Thus the active substance release decreases and the estrogen-containing patch does no longer meet the therapeutic requirements.

Another drawback of systems according to the state of the art is the use of enhancers, this results in a fundamentally undesired additional skin affection including the risk of irritation. Additional disadvantages lie in the expensive construction of these systems

(use of several active-substance-containing layers, use of controlling membranes), generally rendering the finished product unacceptable for the user.

It is accordingly the object of the present invention to avoid the above disadvantages and to provide a stable, i.e., recrystallization-free, estrogen-containing patch or transdermal therapeutic system whose release does not change through storage, wherein the structure is to be designed as thin as possible, and during whose therapeutic application the skin - beyond the active substances estradiol and gestagen - is not treated with skin affecting substances (enhancers).

Most surprisingly, it turned out that this object is achieved by the fact that the estrogen-containing pressure sensitive adhesive is mainly composed of esters of colophony.

In this connection it is of advantage that a styrene-isoprene block copolymer and hydrogenated resin acids or their derivatives are additionally used in the active layer which, for example, comprises a therapeutically required quantity of the active substances estradiol and levonorgestrel.

A combination of the two inactive ingredients, the styrene-isoprene block copolymer serving as cohesive component, and the hydrogenated resin acids or their derivatives serving as tackifying substances, not only results in a rubber adhesive with good tackiness and cohesiveness but also provides excellent biopharmaceutical properties, in particular good skin tolerance and permeation capability, and avoids recrystallization of the active substances.

Thus, the present invention relates to a transdermal therapeutic system for the controlled release of estradiol or its

pharmaceutically acceptable derivatives alone or combined with gestagens, consisting of a backing layer, an active-substance-containing reservoir which is connected thereto and is produced by using pressure sensitive adhesives, and a removable protective layer, with the pressure sensitive adhesive comprising esters of colophony and inactive ingredients.

Examples of esters of colophony include, for example, methyl esters, the glycerol ester, the pentaerythritol ester, the pentaerythritol ester modified with maleic acid, the glycerol ester modified with maleic acid, and the triethylene glycol ester. The proportion of colophony esters in the estradiol-containing pressure sensitive adhesive amounts to 55-92%-wt., preferably 60-90%-wt., and most preferably 70-88%-wt. In addition, the pressure sensitive adhesive may comprise esters of hydrogenated colophony. Particularly preferred esters of colophony include the triethylene glycol ester, the glycerol ester, and the pentaerythritol ester of hydrogenated colophony.

According to another embodiment, the estradiol-containing pressure sensitive adhesive may additionally comprise polymers selected from the group consisting of styrene-butadiene-styrene block copolymers, styrene-isoprene-styrene block copolymers, styrene-ethylene-butylene-styrene block copolymers, ethylene-vinyl acetate copolymers, polyvinyl pyrrolidone, cellulose derivatives, and polymers based on acrylic acid and methacrylic acid derivatives. These polymers are contained in the estradiol-containing adhesive mass at a concentration of 6-25%-wt.

The reservoir of the estradiol-containing patch, wherein recrystallization does not occur, comprises estradiol and its pharmaceutically acceptable derivatives alone or in combination with

gestagens at a total concentration of 2-15%-wt., namely at a molar ratio of 1 : 1 to 1 : 10.

The estradiol-containing reservoir may comprise at least one component of the group including anti-ageing agents, plasticizers, anti-oxidants, and absorption improvers. Suitable plasticizers are known to those skilled in the art and are described, for example, in DE 37 43 949. Usually, the proportion of plasticizers in the estradiol-containing reservoir amounts to 0-5%-wt.

In addition, the active-substance-containing reservoir comprises anti-ageing agents at a concentration of 0-1%-wt. These are known to those skilled in the art and described, for example, in DE 37 43 946.

The estradiol-containing reservoir may either be produced from solution or from the melt.

In case the reservoir fails to exhibit sufficient self-tackiness to the skin, it may be provided with a pressure-sensitive adhesive layer or with a pressure-sensitive adhesive edge. This ensures that the transdermal patch adheres to the skin over the whole application period.

A particularly preferred construction of the transdermal estradiol-containing patch is the matrix system wherein, as is generally known, the matrix controls the active substance release which complies with the \sqrt{t} law according to Higuchi. However, this is not to exclude the possibility that particular cases might require the membrane system. In this case, a membrane controlling the active substance release is located between the reservoir and the pressure sensitive adhesive layer.

The thickness of the transdermal patch depends on the therapeutic requirements and may be adapted accordingly. Usually, it ranges from 0.03 - 0.4 mm.

In addition, a preferred application form is a monolithic matrix-type transdermal therapeutic system which consists of a backing layer substantially impermeable to the active substances, the actually active matrix layer (comprising the active substances and inactive ingredients according to the present invention) and of a removable protective layer.

The examples will show that these systems - although having a simpler construction and being made at lower expenditure than those according to the state of the art - have improved and more constant permeation characteristics for both active substances.

Surprisingly, it turned out that such a formulation which is composed of mainly lipophilic and comparatively low-diffusible polymers and resins results in human blood levels which cannot be obtained with systems according to the state of the art at a comparable low expenditure.

Until today, rubber adhesives have been regarded as being less suitable for the release of estradiol to the skin. For example, EP 0 186 019 describes the idea to use rubber adhesives (in this case by adding water-swellaable substances), this is contradicted in EP 0 421 454 (p. 2, line 54 ff.): a sufficient release of estradiol is not given in the case of these low diffusible and only slightly soluble polymers.

Both substances which are essential to use according to the present invention, styrene-isoprene block copolymer and hydrogenated resin acids or their derivatives, have successfully been used for

long as classic base materials of pressure sensitive adhesive patches and they have a good tolerance. The term "hydrogenated resin acids" means compounds derived from the natural product "colophony". Colophony is widely used as a mixture of native resin acids, above all in chemically modified form, in consumer goods, cosmetics, food packages, chewing gum, etc. It is the resinous residue of the raw product turpentine balsam remaining after distilling off turpentine oil; turpentine balsam originates from different pine trees in mainly subtropical-mediterranean climatic zones.

The crude product is a brittle, resinous mass softening at about 73-80°C and having a density of about 1.07 g/ml. The modification of colophony for the purpose of using it in transdermal therapeutic systems serves to stabilize it against the influence of oxygen by hydrogenation and to improve the alkali stability by esterification. Hydrogenation and derivatization, if necessary, render the material more suitable for the intended purpose. Important esters which can be used for the purpose according to the present invention include, for example, glycerol esters, pentaerythritol esters, methyl esters, and other derivatives of hydrogenated colophony well tolerated by the skin.

Synthetic rubber polymers play an important role in the production of transdermal therapeutic systems and wound dressings. Their advantage lies in the fact that the mechanical properties of transdermal therapeutic systems are considerably improved. To this respect, the styrene-isoprene-styrene block copolymers have proved to be particularly suitable. By dividing the polymer chain into a middle block of still mobile long-chain polyisoprene units and the two polystyrene ends as "anchor points", a three-dimensional network is formed in the matrix, this ensures a substantially constant geometry, even during storage. In this connection it is not decisive which molecular weight or which ratio between the

proportion of the styrene domains and the polyisoprene domains really exists. On the contrary, adjusting the correct tackiness and cohesion is the important factor. For example, an increased resin proportion results in an improved tackiness to the skin but also in a softer consistency of the matrix. In general, the proportion of the block copolymer will amount to about one third, the rest remaining after the active substance addition are biocompatible resin derivatives.

Although a single-layer structure of the transdermal therapeutic system exhibits advantages because of the simple function, it is easily possible according to the present invention to provide such a matrix system, e.g., with a thin additional adhesive layer directed towards the skin. Also, for the purpose of obtaining an improved anchoring effect on the backing layer a thin pressure sensitive adhesive layer may be laminated. Such additional layers may consist of a rubber-resin-mixture but also, for example, of acrylic-ester-containing copolymers. They may be used even if not charged with active substances prior to lamination, since a diffusion compensation takes place during short-time intermediate storage of the complete laminate.

The present invention will be illustrated in more detail by the following examples.

Example 1:

73.1 g triethylene glycol ester of hydrogenated colophony
(Staybelite Ester 3E/by Hercules) and
9.8 g glycerol ester of hydrogenated colophony
(Staybelite Ester 10E/by Hercules)

are mixed by kneading at 100°C for 5 minutes. Then 2.5 g of estradiol are added. Kneading is continued for 30 minutes. After heating to 140°C, 14.6 g ethyl cellulose N50NF (by Hercules) are added in portions, and then kneading is continued for 2.5 hours.

In a hot melt coating line (die coating system) the active-substance-containing adhesive mass thus obtained is coated onto a removable protective layer (Hostaphan RN 100, coated on one side with silicone - by Kalle) in such a manner that an active-substance-containing reservoir having a mass per unit area of 80 g/m² results. An impermeable backing layer (polyester sheet, thickness 15 µm) is laminated on this reservoir. Subsequently, active substance patches of 16 cm² are punched.

Example 2:

The manufacture is in accordance with Example 1, with the plasticizer being kneaded together with the two Staybelite esters 3E and 10E.

Examples 3-9:

Manufacture according to Example 1, however with the raw products and quantities as listed in Table 1 (manufacturing formula).

Analytic procedure

The active substance release of the transdermal patches having a size of 16 cm² is determined according to the Rotating bottle-method described in USP XXII in 0.9% salt solution at 37°C.

To measure the mice skin penetration, the skin of hairless mice is placed in the Franz-cell. An estradiol-containing patch having an

area of 2.54 cm² is stuck onto the skin, and the active substance release is measured at 37°C (acceptor medium: 0.9% saline). (literature: Umesh V. Banakar Pharmaceutical dissolution testing (1st edition - 1991)).

The recrystallization testing is carried out visually against the light.

The results are listed in Table 2.

Table 1: manufacturing formula (indications in g)

Ex.	Ethyl cellulose N50NF	Staybelite Ester		Plasticizer Miglyol 812	Estra- diol	Anti- oxidants
		3E	10E			
1	14.6	73.1	9.8	---	2.5	
2	14.3	71.6	9.6	2.0	2.5	
3	10.1	75.4	10.0	2.0	2.5	
4	7.7	77.5	10.3	2.0	2.5	
5	14.3	71.6	9.5	2.0	2.5	0.1 BHT
6	14.3	71.6	9.5	2.0	2.5	0.1 BHA
7	14.3	71.6	9.5	2.0	2.5	0.1 BHT:BHA = 1 : 1
8	14.3	71.6	9.6	2.0 isopropyl palmitate	2.5	
9	14.3	71.6	9.5 ⑩	2.0	2.5	

BHT = butyl hydroxytoluene

BHA = butyl hydroxyanisole

⑩ = Foral 105 (pentaerythritol ester of hydrogenated colophony)

Table 2: Results of Analysis

Ex.	Estradiol content $\mu\text{g}/16\text{cm}^2$	In-vitro-release $\mu\text{g}/16\text{cm}^2 \cdot 24\text{h}$	Mice skin penetration $\mu\text{g}/16\text{cm}^2 \cdot 24\text{h}$	Recrystallization
1	3200	614	225	no
2	3200	1240	300	"
3	3200	722	235	"
4	3200	713	268	"
5	3200	624	228	"
6	3200	624	249	"
7	3200	620	205	"
8	3200	686	232	"
9	3200	686	232	"
acc. to DE 3933460	3200	2400	125	considerable

The Table shows that a considerably improved penetration through the mice skin is obtained, as evidenced by the comparative example under DE 3933460. Analogously, there is no recrystallization in the Examples according to the present invention.

Example 10:

1.0 g 17- β -estradiol

1.3 g levonorgestrel

60.0 g Cariflex^R TR 1107 (styrene-isoprene-styrene block copolymer), 138.0 g Foral^R 85 (thermoplastic ester resin of colophony derivatives)

200.0 g benzine (boiling range 80-100°C)

are stirred in a cylindrical glass vessel at room temperature until an even suspension results and then coated on a siliconized polyester sheet of 100 μ m thickness in a continuous coating line in such a manner that a layer thickness of 110 g/m² (relative to the solvent-free portion) results. The coating is dried at 40°C, 60°C, 75°C, and 125°C for 3 minutes each. A polyester sheet of 12 μ m thickness is immediately placed on the dry layer without air-bubbles under roll pressure (laminated). Transdermal systems of 20 cm² are obtained by punching using a wad punch.

Example 11: Manufacture of a system according to the invention

1.5 g 17- β -estradiol

1.5 g levonorgestrel

70.0 g styrene-isoprene-styrene block copolymer

150.0 g thermoplastic ester resin of colophony derivatives

are molten and combined by kneading in a heatable kneader at 150°C under nitrogen within 24 h. On a continuous coating line, a polyester sheet of 19 μ m thickness is coated with the melt at a layer thickness of 100 μ m. This may be effected at 140°C in a hot melt coater, or at about 80-100°C by means of an extruder. Subsequently, a siliconized polyester sheet of 150 μ m thickness, pre-coated with 20 g/m² of an acrylic ester copolymer (Durotak^R 280-

2516), is placed on the dried layer (laminated) without air-bubbles and under roll pressure. Transdermal systems of 20 cm² are obtained by punching using a wad punch.

C L A I M S

1. Recrystallization-free estradiol-containing patch in the form of an active substance-containing transdermal therapeutic system for the controlled release of estradiol or its pharmaceutically acceptable derivatives, alone or in combination with gestagen, comprising a

backing layer, an active-substance-containing reservoir which is bonded thereto and produced by using pressure sensitive adhesives, and a removable protective layer,

characterized in that

the estrogen-containing pressure sensitive adhesive comprises esters of colophony at a proportion of 55-92%-wt.

2 . The transdermal therapeutic system according to claim 1 characterized in that the pressure sensitive adhesive comprises esters of colophony at a proportion of 60-90%-wt.

3 . The transdermal therapeutic system according to claim 1 characterized in that the pressure sensitive adhesive comprises esters of colophony at a proportion of 70-88%-wt.

4 . The transdermal therapeutic system according to claim 1 comprising the active substances estradiol and levonorgestrel, characterized in that the active layer of the system comprises a styrene-isoprene block copolymer and hydrogenated resin acids or their derivatives in addition to the active substances.

5. The transdermal therapeutic system according to any one of claims 1 to 4 characterized in that esters of colophony are selected from the group consisting of methyl ester, glycerol ester, pentaerythritol ester, pentaerythritol ester modified with maleic

acid, glycerol ester modified with maleic acid, and triethylene glycol ester.

6. The transdermal therapeutic system according to claim 1 or 2 characterized in that the concentration of estradiol in the active layer amounts to between 0.2 and 2 percent by weight, preferably between 0.7 and 1.4 percent by weight.

7. The transdermal therapeutic system according to claim 1 or 2 characterized in that the concentration of levonorgestrel in the active layer amounts to between 0.1 and 1.6 percent by weight.

8. The transdermal therapeutic system according to one or several of the preceding claims characterized in that the layer thickness of the active layer amounts to between 30 and 300 μm , preferably between 70 and 120 μm .

9. The transdermal therapeutic system according to one or several of the preceding claims characterized in that the proportion of styrene-isoprene block copolymer in the active layer amounts to 10 to 45 percent by weight, preferably 15 to 33 percent by weight.

10. The transdermal therapeutic system according to one or several of the preceding claims characterized in that it comprises one or both of the combination partners, levonorgestrel or estradiol, partially in suspension.

11. The transdermal therapeutic system according to one or several of the preceding claims characterized in that part of the estradiol is present in the transdermal therapeutic system in the form of estradiol crystals, with the estradiol crystals substantially consisting of precipitated estradiol anhydrate.

12. The transdermal therapeutic system according to one or several of claims 1 to 11 characterized in that the pressure sensitive adhesive comprises esters of hydrogenated colophony.

13. The transdermal therapeutic system according to one or several of claims 1 to 12 characterized in that the pressure sensitive adhesive comprises polymers.

14. The transdermal therapeutic system according to one or several of claims 1 to 13 characterized in that the pressure sensitive adhesive comprises polymers at a concentration of 6-25%-wt. and that these are selected from the group consisting of styrene-butadiene-styrene block copolymers, styrene-isoprene-styrene block copolymers, styrene-ethylene-butylene-styrene block copolymers, ethylene-vinyl acetate copolymers, polyvinyl pyrrolidone, and cellulose derivatives, as well as polymers based on acrylic acid and methacrylic acid derivatives.

15. The transdermal therapeutic system according to one or several of claims 1 to 14 characterized in that the reservoir comprises estradiol or its pharmaceutically acceptable derivatives alone or in combination with gestagens at a concentration totalling 2-15%-wt., that is at a molar ratio of 1 : 1 to 1 : 10.

16. The transdermal therapeutic system according to one or several of claims 1 to 15 characterized in that the reservoir comprises at least one component from the group consisting of anti-ageing agents, plasticizers, antioxidants, and absorption improvers, with the plasticizer being contained at a concentration of 0-5%-wt. and the anti-ageing agent being contained at a concentration of 0.1%-wt.

17 . The transdermal therapeutic system according to one or several of claims 1 to 16 characterized in that the pressure sensitive adhesive is a solvent-based pressure sensitive adhesive.

18 . The transdermal therapeutic system according to one or several of claims 1 to 17 characterized in that the pressure sensitive adhesive is a hot-melt pressure sensitive adhesive.

19 . The transdermal therapeutic system according to one or several of claims 1 to 18 characterized in that the reservoir consists of several layers.

20 . The transdermal therapeutic system according to one or several of claims 1 to 19 characterized in that the reservoir is provided with an additional pressure sensitive adhesive layer or with a pressure sensitive adhesive edge.

21 . The transdermal therapeutic system according to one or several of claims 1 to 20 characterized in that a membrane which controls the active substance release is located between the reservoir and the pressure sensitive adhesive layer.

22 . A process for the production of a transdermal therapeutic system as defined in any one of the preceding claims, characterized in that it comprises the following steps:

kneading the mixture of esters of colophony at an elevated temperature until homogenization, incorporating active substance(s) and at least one polymer at the solution temperature, coating a removable protective layer with the active-substance-containing adhesive mass after homogenization, and laminating the backing layer.

23 . The use of the active-substance-containing patch according to any one of claims 1 to 21 for therapeutic purposes in human and veterinary medicine.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
22 April 2004 (22.04.2004)

PCT

(10) International Publication Number
WO 2004/032900 A1

- (51) International Patent Classification⁷: **A61K 9/127**, (74) Agent: MAIWALD, Walter; Maiwald Patentanwalts GmbH, Elisenhof, Elisenstrasse 3, 80335 München (DE).
31/195
- (21) International Application Number: PCT/EP2003/011202
- (22) International Filing Date: 9 October 2003 (09.10.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/417,874 11 October 2002 (11.10.2002) US
10/357,617 4 February 2003 (04.02.2003) US
10/357,618 4 February 2003 (04.02.2003) US
- (71) Applicant (for all designated States except US): IDEA AG [DE/DE]; Frankfurter Ring 193a, 80807 München (DE).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): CEVC, Gregor [DE/DE]; Tassilostrasse 3, 82131 Gauting (DE). VIERL, Ulrich [DE/DE]; Milbertshofenerstrasse 52, 80807 München (DE).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 2004/032900 A1

(54) Title: AGGREGATE WITH INCREASED DEFORMABILITY, COMPRISING AT LEAST THREE AMPHIPATS, FOR IMPROVED TRANSPORT THROUGH SEMI-PERMEABLE BARRIERS AND FOR THE NON-INVASIVE DRUG APPLICATION IN VIVO, ESPECIALLY THROUGH THE SKIN

(57) Abstract: The application describes combinations of at least three amphipatic substances forming aggregate suspensions in a polar liquid. Judicious choice of system components, which differ at least 2-times to 10-times in solubility, ensures said aggregates to have extended, unusually adaptable surfaces. This is probably due to simultaneous action on said aggregates of at least two more soluble substances amongst said three system components, at least one of which is an active ingredient and preferably a drug; the third component, alternatively, can take the role of a drug. The application further deals with the use of said combinations in pharmaceutical preparations capable of transporting drugs into the body of warm blood creatures. This is made possible by the drug loading capability of said aggregates with the highly flexible and deformable coating, which renders the resulting drug carriers highly adaptable. The application finally reveals suitable methods and favourable conditions for carrier manufacturing and application. The application also describes novel formulations of nonsteroidal anti-inflammatory drugs (NSAIDs) based on complex aggregates with at least three amphipatic components suspended in a suitable, e.g. pharmaceutically acceptable, polar liquid medium.

Aggregate with increased deformability, comprising at least three amphipats, for improved transport through semi-permeable barriers and for the non-invasive drug application in vivo, especially through the skin

5

Field of Invention

The invention relates to aggregates with extended surface (extended-surface aggregates, ESAs) with increased deformability and improved barrier
10 penetration capability, said ESAs being suspendable in a suitable liquid medium and comprising at least three amphipats (amphipatic components) and being capable to improve the transport of actives through semi-permeable barriers, such as the skin, especially for the non-invasive drug application in vivo by means of barrier penetration by such aggregates. The
15 three amphipats include at least one membrane forming compound (MFC), which can form the membrane of said ESAs, and at least two membrane destabilising compounds (MDC₁ and MDC₂) differentiated by their capability of forming smaller aggregates (with no extended surfaces) by either themselves or else in combination with each other *and/or* characterized by
20 their relatively high solubility in said suitable liquid medium. The ESAs are loaded with at least one biologically active compound, which can be one of the at least three amphipats.

The invention relates also to preparations comprising extended surface
25 aggregates (ESAs), that can penetrate barriers even when the typical ESAs radius (when an ESA is considered to be spherical) is at least 40% (and preferably at least 50% or even more) greater than the average radius of a pore in the barrier before and after the ESAs have penetrated the barrier.

30 This invention deals also with novel formulations of nonsteroidal anti-inflammatory drugs (NSAIDs) based on complex, extended surface aggregates comprising at least three amphipatic components. One of these components is capable of forming stable, large bilayer membranes on its own. The other at least two amphipatic components, including an NSAID,

- 2 -

tend to destabilise such membranes. Said aggregates are normally suspended in a suitable, e.g. pharmaceutically acceptable, polar liquid medium, which also affects NSAID ionisation. The selection of the second amphipatic membrane destabilising component, which is typically a (co)surfactant, can boost the deformability of the resulting mixed extended surface aggregates. This effect may be supported by judicious choice of the other system components. The invention enables an improvement of barrier penetration and drug delivery by such aggregates. The invention also teaches how to select the most appropriate NSAID concentration, the right total amphipat concentration and, in case, amphipat ionisation in the resulting mixed aggregate suspension. The invention further relates to the preparation and application of the resulting suspension in pharmaceutical formulations, with a focus on epicutaneous application on, or less frequently in, warm blooded creatures.

15

Background Information

Administration of active ingredients frequently is limited by natural barriers, such as the skin, which prevent adequate absorption of the active molecules due to the low barrier permeability for such ingredients.

20

Availability and use of preparations that can overcome this barrier impermeability problem and allow non-invasive active ingredient administration would be advantageous in many cases. In humans and animals, for example, a percutaneous administration of such preparations would protect the active ingredients against decomposition in the gastrointestinal tract and possibly would result in a modified, therapeutically attractive distribution of the agent in the body; such non-invasive administration could also affect the pharmacokinetics of the active ingredient and permit less frequent and/or simpler disease treatment (G. Cevc. Exp. Opin. Invest. Drugs (1997) 6: 1887-1937.). In the case of plants, improved

25

30

- 3 -

penetration through or into the cuticle could lower the concentration of active ingredient that is required for the desired effect and, in addition, could significantly decrease contamination of the environment (Price, C.E. (1981) in: The Plant Cuticle (D.F. Cutler, K.L. Alvin, C.E. Price, Publisher),
5 Academic, New York, pp. 237252).

Many methods for increasing the skin permeability have been discussed (see, for example, G. Cevc, 1997, *op. cit.*). Most prominent are jet injection (for a classical review see Siddiqui & Chien Crit. Rev. Ther. Drug. Carrier
10 Syst. (1987) 3: 195-208), the use of electrical (Bumette & Ongpipattanakul J. Pharm. Sci. (1987) 76: 765-773) or accoustic (Vyas et al., J Microencapsul (1995) 12: 149-54) skin perturbation or else the use of chemical additives, such as certain solvents or surfactants. Such chemicals generally act as the skin permeation enhancers by increasing the partitioning and/or diffusivity of
15 the active ingredient in the skin lipids.

Most often used permeation enhancers are non-ionic short or long-chain alcohols and uncharged surfactants etc., anionic materials (particularly fatty acids), cationic long-chain amines, sulfoxides, as well as various amino
20 derivatives, and amphoteric glycines and betaines. None of these, however, solves the problem of active ingredient transport through the skin or mucous barrier to general satisfaction.

An overview of the measures, which have been used for the purpose of
25 increasing active ingredient penetration through plant cuticles, is summarised in the work of Price (1981, *op. cit.*).

Epidermal use of one or several amphipatic substances in the form of a suspension or an O/W or W/O emulsion, has also brought about too little
30 improvement. An extensive review written by G. Cevc (1997, *op. cit.*) explains why liposomes, at best, can modify drug retention time or stability on the skin and or improve transcutaneous drug transport by partly occluding the skin surface. Japanese patent application JP 61/271204 A2 (86/27 1204)

provides an example for a stabilizing effect of liposomes on the skin, relying on hydroquinone glucosidal as stabilizing material.

5 The use of lipid vesicles loaded with an active ingredient combined with a gel-forming agent in the form of "transdermal patches" was proposed in WO 87/1938 A1. However, the ability of the active ingredient to permeate the skin was not appreciably increased. Massive use of permeation -promoting polyethylene glycol and of fatty acids, together with lipid vesicles, was required by Gesztes and Mezei (1988, Anesth. Analg. 67,1079 -1081) to
10 attain only a moderate local analgesia with lidocaine-containing formulations applied for several hours under occlusion on the skin.

United States Patent 6.193,996 describes a pressure sensitive skin adhesive that uses skin permeation enhancers. European Patent applications EPA 102
15 324 and EPA 0 088 046 and US patent US 4,619.794, all by H. Hauser, describe methods for preparing unilamellar vesicles, using a single membrane destabilising component. The vesicles may be used as carriers for different drugs. However, such vesicles are not used on the skin or for transport through semi-permeable barriers. European Patent application EPA
20 0 152 379 by Muntwyler and Hauser similarly describes the preparation of unilamellar vesicles. However, these vesicles often need to be separated from the residual multilamellar liposomes, facilitated by the presence of charged drugs, for final use of the former for treating the human or animal body. The authors also point to the potential need to neutralize the drug
25 during vesicle preparation to obtain the desired unilamellar liposomes. Further, such vesicles are not used for transport of drugs through a semi-permeable barrier.

European patent EP 0 475 160, corresponding US patent 6,165,500 and
30 Canadian patent 2,067,754, all with the title "Preparation for the application of agents in mini-droplets", describe special preparations related to the suspensions described in this application. These documents report the use of different agents associated with minuscule droplets or, in particular, with the

vesicles consisting of one or a few membrane-like amphiphile assemblies for overcoming semi-permeable barriers including the skin. These references describe preparations having a single membrane destabilising component. WO 98/17255 and AU 724218, likewise, describe vesicles for the transport of
5 a variety of drugs through the skin.

In two relatively early reports on dermal liposomal tetracaine (Gesztcs A, Mezei M. "Topical anesthesia of the skin by liposome-encapsulated tetracaine." *Anesth. Analg.* (1988), 67:1079-1081) and lidocaine (Foldvari M,
10 Gesztcs A, Mezei M. "Dermal drug delivery by liposome encapsulation: clinical and electron microscopic studies." *J Microencapsul* (1990), 7:479-489), Mezei's group reported anaesthetic performance of such locally used drugs and corresponding autoradiography data. Drug was found in the epidermis and in dermis of humans and guinea pigs when the skin was
15 treated under an impermeable (occlusive) coating with the liposome - encapsulated anaesthetics. The formulations always contained multilamellar soybean phosphatidylcholine vesicles. However, the reports demonstrate no liposome-mediated drug transport through the skin. (Foldvari M. "In vitro cutaneous and percutaneous delivery and in vivo efficacy of tetracaine from
20 liposomal and conventional vehicles." *Pharm Res* (1994) 11: 1593-1598) and with an additional oily ingredient (Foldvari M. "Effect of vehicle on topical liposomal drug delivery: petrolatum bases." *J Microencapsul* (1996), 13:589-600). This conclusion is supported by the fact that the reported maximum transported drug dose (5.3%) was more than 20-times higher than the
25 reported transported lipid dose (0.2%) (Foldvari, 1994). Further, Foldvari's formulations evidently were not optimised for adaptability but rather for best drug retention/release.

P. Gonzalez, M. E. Planas, L. Rodriguez, S. Sanchez, and G. Cevc in an
30 article on "Noninvasive, percutaneous induction of topical analgesia by a new type of drug carriers and prolongation of the local pain-insensitivity by analgesic liposomes" (*Anesth. Analg.* (1992), 95: 615-621) report the results of investigations with surfactant-containing formulations, typically loaded with

- 6 -

lidocaine (2%, as a free base) in a mixed lipid 4-8% suspension (w/v). Lipid aggregates were prepared from a 4/1 mol/mol phosphatidylcholine/sodium cholate mixture, starting with an ethanolic lipid solution (7-3 w-% EtOH in the final product) for easier manufacturing. However, all the tested suspensions
5 were reported by Planas et al. to be unstable. Further, Planas et al. failed to disclose how a stable drug formulation could be prepared, which would be suitable for transdermal drug delivery.

Peters and Moll (1995) ("Pharmacodynamics of a liposomal preparation for
10 local anaesthesia". *Arzneimittelforschung* (1995), 45:1253-6, describe permeation of a topically applied drug through the skin. The permeation is enhanced by ethanol, is based on diffusion, and is achieved under occlusion.

Carafa and colleagues describe the use of surfactant-based, phospholipid-free vesicles (Carafa et al., 2002 ("Lidocaine-loaded non-ionic surfactant
15 vesicles: characterisation and in vitro permeation studies." *Int J Pharm* (2002), 231 :21-32). However, such vesicles do not simultaneously include both a *MFC* and a *MDC*, and are unsatisfactory.

20 The current state of the art in particular in NSAID delivery through the skin is transdermal drug diffusion, which is proportional to the drug concentration on the skin and inversely proportional to the skin barrier resistance, which is tantamount to saying that diffusion is proportional to the skin permeability.

25 Solubility of typical NSAIDs is in the range 1 ~g/ml to between 0.5 mg/ml and 10 mg/ml for the pH range between 1 and 7.5. This corresponds to a few μ M and up to a few tens of mM, high values being always measured in least acidic solutions ($\text{pH} \gg \text{pKa}$) where NSAIDs are partly or completely ionised, the solubility at $\text{pH} \ll \text{pKa}$ always being very low. To maximise diffusive
30 NSAID transport through the skin one should therefore always use the highest tolerable *pH*, which can exceed the value of 9.

- 7 -

Taken the limitations of maximum NSAID solubility, attempts have been made to improve NSAID permeation (diffusion) through the skin by using permeability or permeation enhancers. Permeability enhancers increase NSAID flux through the barrier for a given drug concentration, but do not
5 much affect the depth of drug distribution. Further, use of conventional lipid formulations on the skin does not affect this limitation.

For example, Henmi et al. 1994 (Chem Pharm Bull 42:651-655) used three different NSAIDs (ketoprofen, flurbiprofen and ibuprofen) in an oily gel,
10 formed by hydrogenated soybean phospholipids (which forms very stiff membranes) and applied the preparation on the skin. The conclusion was that such lipids have no permeation enhancing effect for the skin but rather solubilise the test drug.

15 Burnham et al. 1998 (Clin J Sport Med 8:78-81) used a block co-polymer of polyethylene and an unspecified polypropylene glycol (pluronic), which generally is a poor membrane destabilising amphipat, to apply an NSAID on the skin. An unspecified lecithin based liposomal organo-gel (PLO) was furthermore used three times daily for one week, followed by a weekly
20 "washout" period without using the gel. The authors noted that only a thin tissue layer under the skin was treated, thus implying that any apparently positive result could be due to free drug diffusion from PLO through the skin. Organo-gel consequently has served as merely a superficial reservoir.

25 Vyas et al. (J Microencapsul 12:149-54, 1995) incorporated diclofenac into multilamellar, 1-5 μm large liposomes at $pH = 7.4$ that were applied on the skin under different conditions. The resulting systemic drug availability was then studied. The resulting mixed lipid vesicles were incorporated in an ointment base and were applied on the skin of rats. However, skin poration
30 by ultrasound was required to achieve any substantial transdermal delivery of

- 8 -

the drug, and most of the tested NSAID was typically found at the site of application.

- 5 Schramlova et al. (Folia Biol (Praha) 43:195-199,1997) associated ibuprofen with liposomes prepared from soybean phospholipid supplemented with 10 rel-% cholesterol, the knowledge in the art being that the latter is a membrane stiffening agent. The formulation with a $pH = 7.4$ was injected intramuscularly or applied under occlusion on the skin. NSAID from lipid vesicles occasionally decreased the rat leg edema slightly, but not 10 significantly, better than the drug from a conventional cream but less than an NSAID injection. This paper therefore teaches the use of a membrane stabilising component (cholesterol) rather than of a membrane destabilising component.
- 15 Saunders et al. (J Pharm Pharm Sci 2:99-107,1999), studying the skin permeation enhancement, also used liposomal structures of unspecified composition and morphology, which were claimed to be present in the MZL lotion and in a comparator gel (both prepared by Meyer Zall Laboratories (MZL)), and loaded with sodium diclofenac. The presence of oil in the 20 oil/water base in the MZL formulation, which diminishes lipid aggregate deformability, and occludes the skin, if nothing else precluded efficient drug delivery by vesicle through the skin.
- 25 Calpena et al. (Arzneimittelforschung 49:1012-1017,1999) studied diclofenac permeation through human skin from 6 semisolid formulations containing 1 % drug in a complex mixture of gel-forming materials combined with lecithin (2.5% of unspecified quality) and cholesterol (0.5%). However, the results of the studies suggest that use of lipid vesicles is not beneficial (Calpena et al., 1999).

- 9 -

Skin permeability data for ibuprofen lysinate was studied, showing practically equal permeability rates for the drug in solution or in mixed micelles (containing soy-bean phosphatidylcholine) and nearly 3-times lower rate for the corresponding liposomal dispersion (Stoye et al., 1998 (Eur J Pharm Biopharm 46:191-200). Liposomes therefore were concluded to be useless in terms of supporting transdermal drug transport in the described system.

Summary of the Invention

Applicants have discovered that incorporation of a surfactant into a bilayer membrane that is built from another less soluble amphipat, such as a phospholipid, can increase the flexibility of the resulting complex membrane. This promotes the capability of complex aggregates in the form of droplets covered by the bi-component membranes to cross pores in a semi-permeable barrier that otherwise would prevent comparably large aggregates from crossing. Further, the use of aggregates with highly deformable membrane coating can mediate agent transport into and/or across mammalian skin. This can be achieved by selecting a surfactant, which is a membrane destabilising component (= *MDC*), and a less soluble amphipat, which is the membrane forming component (= *MFC*), so as to maximize the mixed membrane flexibility and the mixed aggregate stability. Further the surfactant can be selected to increase bilayer membrane adaptability. Patent applications by applicant, especially WO 92/03122 and WO 98/172550 describe basic requirements for the use of lipid/surfactant mixtures for transbarrier transport.

It is an objective of the invention to provide preparations that can transport active ingredients through a barrier in the form of vesicles or other extended surface aggregates (ESAs) comprising said actives, said preparations having improved permeation capability through semi-permeable barriers.

It is a further aspect of the invention to provide a preparation based on a combination of at least one first (membrane forming component *MFC*), at least one second (membrane destabilising component *MDC*), and at least one third (membrane destabilising component *MDC*) amphipatic component
5 suspended in a suitable liquid medium in the form of corresponding mixed amphipat extended surface aggregates (ESAs) with one or a few bilayer-like, mixed amphipat coating(s), wherein said ESAs formed by a combination of all three said components have surfaces in contact with said liquid medium, that are at least 50% more extended, on the average, than the typical
10 surfaces of aggregates comprising the said at least one second and at least one third amphipatic component alone, at the same concentrations and, in case, after adjustment for physico-chemical effects of the absence of said first amphipatic compound (*MFC*).

15 A further aspect of the invention is to provide suspensions of extended surface aggregates in a liquid medium comprising: at least one first membrane forming component (*MFC*); at least one second membrane destabilising component (*MDC*); at least one third membrane destabilising component (*MDC*), the third component typically being a drug, such that said
20 complex extended surface aggregates (ESAs) can penetrate intact mammalian skin and thus increase drug concentration in the skin and/or increase the reach of drug distribution below the skin, in comparison with the result of the same drug application in a solution on the skin. In a special version of said suspensions, said extended surface aggregates are
25 membrane-enclosed, liquid-filled vesicles, said first component is a membrane-forming lipid, and said second and third components are membrane-destabilising components.

Another aspect of the invention provides a combination of at least one first
30 (membrane forming, component *MFC*), at least one second (membrane destabilising component *MDC*), and at least one third (membrane destabilising component *MDC*) amphipatic component suspended in a suitable liquid medium in the form of mixed amphipat extended surface

- 11 -

aggregates (ESAs) with one or a few bilayer-like, mixed amphipat coating(s), wherein the

5 - said at least one first substance has a tendency to self aggregate and is at least 10 times less soluble in said liquid medium than said at least one second and said one third substance, allowing the first to form extended surfaces,

10 - said at least one second substance is at least 10-times more soluble than said at least one first substance in said liquid medium and, on its own, tends to form or supports the formation of surfaces, that are at least 2-times less extended than the surfaces containing the at least one first substance alone,

15 - said at least one third substance being also at least 10-times more soluble in said liquid medium than the first substance and optionally forms self-aggregates with aggregation numbers at least 10-times smaller than that of self-aggregates of said first substance; and

20 - said extended surfaces comprising said at least one first, at least one second and at last one third substance, in equilibrium, have at least 50% more extended surfaces than the surfaces formed by the at least one second or one third substance alone, at the same concentration and, in case, after adjustment for physico-chemical effects of the absence of
25 said first amphipatic compound (*MFC*).

Yet another aspect of the invention is a preparation based on a combination of at least one first (membrane forming component *MFC*), at least one second (membrane destabilising component *MDC*), and at least one third
30 (membrane destabilising component *MDC*) amphipatic component suspended in a suitable liquid medium in the form of corresponding mixed aggregates with an extended surface (ESAs) with one or a few, preferably bilayerlike, mixed amphipat coating(s), wherein said *MFC* alone forms

- 12 -

extended-surface aggregates with aggregation number of at least 5000, and preferably more than 10.000, and both *MDCs* alone and the combination of both *MDCs* form smaller aggregates with no really extended surface and aggregation number below 5000, and preferably below 1000 in contact with
5 said suitable liquid medium.

All compositions according to the present invention comprising three amphipatic compounds which together form extended surface aggregates either have a defined solubilization point, or do comprise more than 0.1 mol%
10 of the solubilizing amount of those components which at higher concentrations would solubilize the extended surface aggregates.

All embodiments of the invention are useful in preparations for the application, administration or transport of at least one active ingredient which
15 can be amongst said three substances, especially for medicinal or biological purposes, into and through barriers and constrictions, such as the skin of warm blood creatures or the like.

Preferably the adaptability of extended surface comprising all three said
20 amphipatic components to ambient stress exceeds by at least 20% or by at least twice the standard deviation of a typical measurement (whichever is smaller) the adaptability of the extended surface comprising the at least one first and the at least one second amphipatic component used at the corresponding concentrations or the adaptability of the extended surface
25 comprising the at least one first and the at least one third amphipatic component at corresponding concentrations, whichever is smaller.

The adaptability can be expressed as the inverse value of the p^* value. This specific p^* value is typically higher than 50%, often is around 60% and
30 preferably is 57% of P_{max} -value.

- 13 -

We have further found, unexpectedly, that various combinations of at least two amphipatic components one of which is an NSAID, which can substantially destabilise a lipidbased, otherwise stable extended surface aggregate, typically in the form of a bilayer membrane, can synergistically increase the resulting at least three component aggregate adaptability. In parallel, the aggregate (membrane) shape deformability is synergistically augmented. Consequently, the flux of such aggregate suspension through narrow pores is increased and/or the characteristic pressure that drives certain flux through the corresponding porous barrier is lowered.

10

The capability of said at least three-component aggregates to move through a semi-permeable barrier is thus facilitated. This finding is surprising given that the droplets covered by a bi-component bilayer membrane already have an appreciable barrier crossing capability compared to droplets enclosed by a simple lipid bilayer.

15

The increase of adaptability of said extended surface aggregates with at least three amphipatic components and/or the lowering of the pressure that is needed to make such aggregates move through a biological barrier has important, and unexpected, practical consequences. Specifically, when said aggregates are applied on the skin, as an example for a biological semi-permeable barrier, the transport of the aggregate associated NSAIDs through such barrier is increased and reaches further. The latter observation is explicable in terms of differential clearance in the superficial skin layers, where cutaneous blood drainage resides, of the drug, which can enter directly into blood capillaries, and of drug-loaded aggregates, which are too big to enter such capillaries. This means that NSAID carriers move further than the drug from solution, allowing deeper tissues to be treated with NSAIDs under the drug application site on the skin. Convincing evidence for this is given in one of Practical Examples. Such finding is not expected taken

20

25

30

- 14 -

that simple NSAID phospholipid combinations already ensure better and deeper drug transport through the skin than conventional preparations based on NSAID solutions.

- 5 Further objectives and advantages of the instant invention will become apparent from the following description of preferred embodiments, which include a best mode preparation.

10 In the present description, the general terms employed hereinbefore and hereinafter have the following meanings.

The term "**aggregate**" denotes a group of more than just a few amphipats of similar or different kind. A small aggregate, as used in the context of this invention, has an aggregation number $n_a > 3$, that is, contains at least 3
15 molecules, but does not exceed $n_a < 5000$ or more preferably $n_a < 1000$, that is, contains no more than 5000 or 1000 molecules. The "extended surface aggregate (ESA)", "an aggregate with extended surface", a "vesicle" or an "extended surface" as used in the context of this invention, all have aggregation numbers ≥ 5000 , that is, contain a minimum of 5000 molecules,
20 and most often are characterized by an even higher aggregation number, that is, contain an even higher number of molecules. Preferred ESAs have aggregation numbers of $n_a > 10000$ and even more preferably $n_a > 50000$. For a preparation containing aggregates, the reference will always be made to the average aggregation number or to the average number of molecules
25 per aggregate, except if indicated otherwise. The term "aggregation number" equals the number of molecules which together form an aggregate. Corresponding methods of n_a determination are well known in the art.

30 When a lipid aggregate is water filled and surrounded with at least one membrane it is called a lipid vesicle. The membrane as defined in this description is a mixture of at least three amphipats ($MFC + MDC_1 + MDC_2$) preferably in the form of a bilayer; a membrane destabilising component

- 15 -

hereby is potentially a *MFC-MDC* combination (i.e. a mixed amphipat associate).

5 The aggregates of the invention are coated with one half, one, or several bilayers. These may also be called mixed amphipat coating(s), and correspond to a lipid monolayer, bilayer or oligolayers, respectively.

10 For a solid aggregate with the surface comprising only one layer of molecules (a monolayer)" the aggregate surface $S_{aggregate}$ given by the product of aggregate number and the exposed single molecule surface $S_{molecule}$:

$$S_{aggregate} = n_a S_{molecule}$$

15 $S_{molecule}$ can either be measured directly, e. g. in a Langmuir trough or with a diffractometric or reflectometric method, or else can be calculated with any suitable computer model (e.g. HyperChem).

An aggregate with a bilayer coating has a surface area only half as large:

20
$$S_{aggregate}(bilayer, n_a) = 0.5 S_{aggregate}(monolayer, n_a).$$

"Aggregate radius" r_a for a spherical aggregate is proportional to the square root of the aggregate surface:

25

$$r_{aggregate} = (S_{aggregate}/4\pi)^{0.5}$$

other aggregate geometries requiring appropriate formula adaptation.

30 A "**barrier**" in the context of this invention is (as in, for example, EP 0 475 160 and WO 98/17255) a body with through-extending narrow pores, such narrow pores having a radius which is at least 25% smaller than the radius of the ESAs (considered as spherical) before said ESAs permeate through such

pores.

The term "**narrow**" used in connection with a pore implies that the pore radius is significantly, typically at least 25%, preferably at least 30% smaller than the radius of the entity tested with regard to its ability to cross the pore. The necessary difference typically should be greater for the narrower pores. Using 25% limit is therefore quite suitable for >150 nm diameter whereas > 100% difference requirement is more appropriate for the smaller systems, e.g. with < 50 nm diameter. For diameters around 20 nm, aggregate diameter difference of at least 200% is often required.

The term "**semipermeable**" used in connection with a barrier implies that a solution can cross transbarrier openings whereas a suspension of non-adaptable aggregates (large enough for the above definition of "narrow" pores to apply, typically 150-200% larger than the diameter of such openings) cannot. Conventional lipid vesicles (liposomes) made from any common phosphatidylcholine in the gel lamellar phase or else from any biological phosphatidylcholine/cholesterol 1/1 mol/mol mixture or else comparably large oil droplets, all having the specified relative diameter, are three examples for such non-adaptable aggregates.

The term "**stable**" means that the tested aggregates do not change their diameter spontaneously or under the transport related mechanical stress (e.g. during passage through a semipermeable barrier) unacceptably, which most often means only to a pharmaceutically acceptable degree. A 20-40% change is normally considered acceptable; the halving or doubling of aggregate diameter is borderline and a greater change in diameter is typically unacceptable. Alternatively and very conveniently, the change in aggregate diameter resulting from pore crossing under pressure is used to assess system stability; the same criteria are then applied as for "narrow" pores, mutatis mutandis. To obtain the correct value for aggregate diameter change, a correction for flux/vortex effects may be necessary. These procedures are described in greater detail in the publication of the applicant in Cevc G.,

Schätzlein A., Richardsen H. (2002) Ultradeformable Lipid Vesicles Can Penetrate the Skin and other SemiPermeable Barriers Intact. Evidence from Double Label CLSM Experiments and Direct Size Measurements. Biochim. Biophys. Acta 1564:21-30.

5

The term "**barrier transport resistance**" describes the resistance of a given barrier to the transport of a given fluid with or without suspended aggregates. Mathematically speaking, this resistance is given by the ratio of transport driving pressure and of transport rate (=flow): resistance = $\Delta p / j_a$. In

10

more qualitative terms, used in some of the examples in this document, barrier resistance is identified with the total fluid volume that can be filtered through a given barrier by certain pressure within given time. Alternatively the pressure needed to achieve certain flux can be used to describe functionally barrier resistance.

15

Barrier transport resistance generally decreases linearly with the number and total area of pores in the given transport obstacle. For relatively small pores the resistance value can also depend on average pore diameter, mainly due to friction/viscosity effects. In addition to this, barrier transport resistance is sensitive to transported fluid / suspension characteristics and thus strongly depends on the suspended particle adaptability and sometimes concentration. In the first approximation, this later sensitivity is due to elastic and viscous loss during transport.

20

25 The term aggregate "**adaptability**" which governs the "tolerable surface curvature" is defined as the ability of a given aggregate to change easily, and essentially reversibly, its properties, such as shape, elongation ratio, and surface to volume ratio. Essential for this invention is the adjustment of aggregate shape and properties to the anisotropic stress caused by pore crossing. Sufficient adaptability implies that an aggregate is able to sustain
30 different unidirectional forces or stress, such as pressure, without significant fragmentation, which defines a "stable" aggregate. If an aggregate passes through a barrier fulfilling this condition the terms "adaptability" and (shape)

"deformability" plus "permeability" are essentially equivalent.

Non-destructing passage of ultradeformable, mixed lipid aggregates through narrow pores in a semi-permeable barrier is thus diagnostic of high
5 aggregate adaptability. If pore radius is two times smaller than the average aggregate radius the aggregate must change its shape and surface-to-volume ratio at least 100% to pass without fragmentation through the barrier. An easy and reversible change in aggregate shape inevitably implies high aggregate deformability and requires large surface-to-volume ratio
10 adaptation. A change in surface-to-volume ratio per se implies: a) high volume compressibility, e.g. in the case of compact droplets containing material other than, and immiscible with, the suspending fluid; b) high aggregate membrane permeability, e.g. in the case of vesicles that are free to exchange fluid between inner and outer vesicle volume.

15 Measuring capability of given aggregate suspension to cross a semi-permeable barrier with narrow pores thus offers simple means for functionally testing aggregate adaptability, as is described in Practical Examples. This capability for suspensions of sufficiently stable aggregates is inversely
20 proportional to the effective barrier transport resistance and, in the first approximation, to vesicle adaptability $a_v = a_a$ (subscripts v and a denoting vesicle and aggregate, respectively). If no other adaptability value is available, the inverse value of barrier transport resistance or $1/p^*$ value, which are defined further in the text, can be used to characterise adaptability
25 of aggregates in a suspension.

The adaptability of a vesicle-like aggregate depends on reversible vesicle membrane permeability and deformability. Lipid bilayer permeability can be assessed by the well established methods, such as the osmotic swelling
30 method that is described in many scientific papers and in Phospholipids Handbook, edited by G. Cevc for Marcel Dekker Publishers (New York, 1993). Less directly and quantitatively, but still telling, vesicle bilayer

- permeability can be checked by comparing the average aggregate diameter before and after pore crossing: vesicle bursting and fragmentation is indicative of aggregate membrane impermeability. In case of lipid vesicles, the latter is identical to lipid bilayer impermeability. Open membrane
- 5 deformability is governed by lipid bilayer flexibility. This quantity is proportional to bilayer bending elasticity and is hence determined by the elastic membrane bending modulus = the elastic curvature modulus of a bilayer = B . The latter parameter can be measured with several methods known in the art, including pipette aspiration measurements, vesicle shape or
- 10 fluctuation analysis, bilayer deformation under stress in an atomic force microscope, etc.. Bilayer curvature elastic energy density of a vesicle with radius r_{ves} is given by $B/2r_{ves}^2$, which shows that most elastic/flexible bilayers, with smallest B -values, are most deformable. For phosphatidylcholine bilayers in the fluid lamellar phase B -value is typically of the order 10^{-19} J.
- 15 This value is at least one order of magnitude higher than the corresponding value determined for a suitable *MDC-MFC* or *MDC- MDC-MFC* mixture, which is $B \sim 5 \cdot 10^{-17}$ J. This explains why the described three-component amphipat mixtures form very flexible bilayers and highly deformable vesicles.
- 20 It is important to realize that any system property that tends to lower aggregate shape adaptability also lowers the likelihood for aggregate motion through the pores with a radius smaller than the average aggregate radius. Incorporation of large incompressible bodies (e.g. oil droplets) into or between the shape-deformable aggregates therefore lowers, if not
- 25 blocks, trans-barrier transport. Incompressibility of aggregate core has similarly negative effect. Aggregates in the form of (lipid) vesicles suspended in and filled with nearly incompressible water must therefore expel some water from vesicle interior during aggregate deformation to attain high/maximum adaptability. Introduction of membrane stiffening
- 30 agents (including cholesterol and other sterols, little polar long chain lipids, etc., as quasi-MFC) into bilayers also lowers the adaptability of the resulting mixed aggregates. Vesicle-like aggregates with many bilayer coatings (= membranes) are also relatively non-adaptable (i.e. have lower

- 20 -

- a_a value, as defined further in the text) and must be pushed with a higher force (i.e. have a higher p^* value, as defined further in the text) through narrow pores than the aggregates with just a few or only one such coating(s). The reasons for this are obvious: in the simplest approximation,
- 5 aggregate adaptability is inversely proportional to the number of bilayers enshrining liquid core of an aggregate. Further system changes that negatively impact on aggregate adaptability can be analyzed in similar fashion.
- 10 If a vesicle can pass through a narrow pore without irreversibly adjusting its diameter to the pore diameter within 50% or even 100% uncertainty range, the vesicle bilayer membrane under terms of this document is declared to be permeable as well as flexible. To assess lipid aggregate adaptability it is therefore useful to employ another aspect of the invention, by using the
- 15 following method:
- 1) measure the flux j_a of aggregate suspension through a semi-permeable barrier (e.g. gravimetrically) for different transport-driving trans-barrier pressures Δp ;
 - 20 2) calculate the pressure dependence of barrier penetrability P for the given suspension by dividing each measured flux value with the corresponding driving pressure value:
- 25
$$P(\Delta p) = j_a(\Delta p) / \Delta p;$$
- 3) monitor the ratio of final and starting vesicle diameter $2r_{ves}(\Delta p) / 2r_{ves,0}$ (e.g. with the dynamic light scattering), wherein $2r_{ves}(\Delta p)$ is the vesicle diameter after semi-permeable barrier passage driven by Δp
 - 30 and $2r_{ves,0}$ is the starting vesicle diameter, and if necessary making corrections for the flow-rate effects;

- 4) align both data sets $P(\Delta p)$ vs. $r_{ves}(\Delta p)/r_{ves,0}$, to determine the co-existence range for high aggregate adaptability and stability; it is also useful, but not absolutely essential, to parameterise experimental penetrability data within the framework of Maxwell-approximation in terms of the necessary pressure value p^* and of maximum penetrability value P_{max} , which are defined graphically in the following illustrative schemes.

Figures 1 to 4 illustrate schematically the physical and molecular principles underlying the abovementioned approach and the mathematical model used to analyse the corresponding experimental data.

It is plausible to sum-up all the contributions to a moving aggregate energy (deformation energy/ies, thermal energy, the shearing work, etc.) into a single, total energy. The equilibrium population density of aggregate's energetic levels then may be taken to correspond to Maxwell's distribution. All aggregates with a total energy greater than the activation energy, $E > E_A$, are finally concluded to penetrate the barrier. The pore-crossing probability for such aggregates is then given by:

$$P(e) = 1 - \operatorname{erf}\left(\sqrt{\frac{1}{e}}\right) + \sqrt{\frac{4}{\pi e}} \cdot \exp\left[-\frac{1}{e}\right],$$

e being dimensionless aggregate energy in units of the activation energy E_A .

It is therefore plausible to write barrier penetrability to a given suspension as a function of transport driving pressure (= driving pressure difference) p (= Δp) as:

$$P(P_{max}, p^*, p) = P_{max} \cdot \left\{ 1 - \operatorname{erf}\left(\sqrt{\frac{p^*}{p}}\right) + \sqrt{\frac{4p^*}{\pi p}} \cdot \exp\left[-\frac{p^*}{p}\right] \right\} \quad (*)$$

- P_{max} is the maximum possible penetrability of a given barrier. (For the aggregates with zero transport resistance this penetrability is identical to the penetrability of the suspending medium flux.) p^* is an adjustable parameter that describes the pressure sensitivity, and thus the transport resistance, of the tested system. (For barriers with a fixed pore radius this sensitivity is a function of aggregate properties solely. For non-interacting particles the sensitivity is dominated by aggregate adaptability, allowing to make the assumption: a_a proportional to $1/p^*$.)
- 10 In a presently preferred embodiment of the invention, the experimental approach to quantitative aggregate adaptability determination is to identify vesicle adaptability value with the inverse pressure difference needed to attain certain predefined, practically relevant fraction of maximum achievable flux-pressure ratio with the vesicle suspension; using 50-60% maximum
- 15 penetrability criterion (P_{max}) gives reasonable results. Specifically, all p^* values given in this document correspond to 57% of P_{max} -value. Adaptability value, up to an uninteresting constant, is then given by the inverse value of the p^* value that corresponds to 57% of the P_{max} -value.
- 20 By making a few more reasonable suppositions one can use the experimentally determined p^* -value to calculate the activation energy E_A for transbarrier transport of adaptable vesicular aggregates. The dominant energetic contribution to the work of bilayer deformation - bilayer elastic energy; bilayer permeabilisation energy, as the case may be - can then be
- 25 deduced from E_A -value. Finally, bilayer elastic energy can be translated into bilayer curvature elastic energy density, which depends on the elastic curvature modulus of bilayer, B , as is explained earlier in the text. Bilayer permeabilisation energy independently can be related to the work needed to break a bilayer membrane, and thus to bilayer lysis tension, assuming that
- 30 elastic energy is much smaller than membrane permeabilisation energy. For

simple lipid vesicles this has been done by the group of B. Frisken (cf. Biophys. J. 74: 2996-3002 (1998) and Langmuir 16: 928--933 (2000)), amongst others. Such detailed analysis is not necessary for optimising aggregate suspensions for transbarrier transport, however, and therefore is
5 not used in the present application.

The "**liquid suspending medium**" or "liquid medium" or "suitable liquid medium" is defined in EP 0 475 160 and in WO 98/17255.

10 An "**amphipat**" (or an amphipatic component) is any substance capable of forming an ESA or of modifying the adaptability of an ESA, when brought into contact with the liquid suspending medium.

For the broadest definition, the amphipats are divided into two subgroups, the
15 "**membrane forming compounds**" (*MFCs*) or "surface building" or "extended surface-forming or "surface-supporting substance", which are capably of forming extended surface aggregates (ESAs), and "**the membrane destabilising compounds**" (*MDCs*). The latter typically render the ESAs formed by the *MFCs* more adaptable.

20 In some aspects the three amphipatic compounds, one *MFC* and two *MDCs* forming the ESAs are then defined that the *MFC* alone forms ESAs, the one *MDC* alone forms small aggregates, the other *MDC* alone optionally forms small aggregates and the combination of both *MDCs* forms small aggregates,
25 in contact with said liquid suspending medium. The ESAs and the small aggregates being defined in terms of aggregation numbers as stated above.

In some aspects the three amphipatic compounds, one *MFC* and two *MDCs* forming the ESAs are then characterised by their solubility in the liquid
30 suspending medium. The *MFCs* are then defined to be less soluble than the

MDCs at least by a factor of 2. In more preferred embodiments the *MFCs* are then defined to be less soluble than the *MDCs* at least by a factor of 10 and in preferred embodiments the solubilities of the two *MDCs* differ at least by a factor of 2. Alternatively or simultaneously the *MFCs* are defined to be less
5 soluble than the *MDCs* at least by a factor of 10, one *MDC* forms aggregates with surfaces that are at least 2 times less extended than the surfaces of aggregates formed by the *MFC* and the other *MDC* forms aggregates with aggregation numbers at least 10 times smaller than the aggregation numbers of aggregates formed by the *MFC*. Yet another possibility is to define *MDC* as
10 molecules, which are typically characterised by hydrophilicity-lipophilicity ratio (HLB) between 10 and 20, even better between 12 and 18 and most preferred between 13 and 17.

In some aspects the *MFC* and *MDCs* are defined to form in the combination
15 of one *MFC* and two different *MDCs* extended surface aggregates with surfaces that are at least 50% more extended, extended meaning larger, on the average than surfaces of aggregates comprising only the two different *MDCs* alone, at the same concentrations and, in case, after adjustment for physico-chemical effects of the absence of said *MFC*.

20

For some aspects a selection or all definitions at once apply.

Within the meaning of the present invention the *MFC* is preferably a lipid and more preferably a phospholipids as defined below.

25

The amphipats within the meaning of the present invention comprise the membrane forming substances and the "edge-active (surface active)" substances also known from EP 0 475 160 and WO 98/17255, but within the limitations defined in the attached claims.

30

- 25 -

The term "**drug**" means a biologically or therapeutically active ingredient, e.g. a medicament. Unless indicated otherwise, the generic names proposed by the world Health Organisation (WHC)) (Recommended International Non-proprietary Names), such as can be found e.g. in the Merck Index, are used
5 for the drugs, which are specified in greater detailed further in the text.

The term "**low**" used in connection with molecular weight of a polypeptide means molar mass below 1500 and the term "intermediate" in similar context implies molar mass between 1500 and 5000.

10

The term "**lower**" used in connection with organic radicals, for example lower alkyl, lower alkylene, lower alkoxy, lower alkanoyl, etc., means that such organic radicals, unless expressly defined otherwise, contain up to and including 7, preferably up to and including 4, carbon atoms.

15

The term "**long**" used in connection with a fatty residue attached to a lipid, a surfactant or a drug implies the presence of 10 to 24 carbon atoms in alkyl, alkenyl, alkoxy, alkenyloxy or acyloxy chains, which individually or together, as the case may be, bear the class name of "fatty chains". Implicitly included
20 in this term, but not further specified in detail, are "fatty chains" with at least one branched or a cyclic, but unpolar or little polar, segment.

The use of square brackets in the text relates to molar concentrations of the substance put between the brackets, except if indicated otherwise.

25

The terms "**surface active**" and "**edge active**" relates to the ability of a certain third compound to change the surface tension and/or interface tension in systems comprising at least two compounds forming a surface or interface.

30

In this specification the terms "**compound**", "**substance**" and "**component**" generally indicate a single chemical species, which needs, however, not to be totally uniform.

- 5 The term "**apparent dissociation constant**" refers to the measured dissociation (i.e. ionisation) constant of a drug. This constant for many drugs, including NSAIDs, is different in the bulk and in the homo- or heteroaggregates. For ketoprofen, the pKa in the bulk is approx. 4.4 whereas the pKa value measured above the drug association concentration is approx. 5, and decreases approximately linearly with the inverse ionic strength of the bulk solution. pKa of ketoprofen bound to lipid bilayers increases with total lipid concentration as well, and is approx. 6 and 6.45 in suspensions with 5 w-% and 16 w-% total lipid in a 50 mM monovalent buffer, respectively. For diclofenac, the pKa in the bulk is around 4, whereas for this drug in lipid bilayers pKa ~ 6.1 was determined. The bulk pKa reported in the literature for meloxicam, piroxicam, naproxen, indomethacin and ibuprofen is 4.2 (and 1.9), 5.3, 4.2-4.7, 4.5, and 4.3 (or in some reports 5.3), respectively.

- The term aggregate "**deformability**" is closely related to the term "**adaptability**".
- 20 Any major change in aggregate shape that does not result in a significant aggregate fragmentation is indicative of sufficient aggregate deformability, and also implies a large change in the deformed aggregate surface-to-volume ratio. Deformability can therefore be measured in the same kind of experiments as is proposed for determining aggregate adaptability, or else can be assessed by
- 25 optical measurements that reveal reversible shape changes.

The term "**NSAID**" (nonsteroidal anti-inflammatory drug) typically indicates a chemical entity which acts as lipooxygenase, cyclooxygenase-1 or cyclooxygenase-2 antagonist.

- 27 -

Examples include salts of substituted phenylacetic acids or 2-phenylpropionic acids, such as alclofenac, ibufenac, ibuprofen, clindanac, fenclo rac, ketoprofen, fenoprofen, indoprofen, fenclofenac, diclofenac, flurbiprofen, pirprofen, naproxen, benoxaprofen, carprofen or cicloprofen; analgesically active heteroarylacetic acids or 2-heteroarylpropionic acids having a 2-indol-3-yl or pyrrol-2-yl radical, for example indomethacin, oxmetacin, intrazol, acemetazin, cinmetacin, zomepirac, tolmetin, colpirac or tiaprofenic acid; analgesically active indenylacetic acids, for example sulindac; analgesically active heteroaryloxyacetic acids, for example benxadac; NSAIDS from oxicame family include piroxicam, droxicam, meloxicam, tenoxicam; further interesting drugs from NSAID class are, meclofenamate, etc.

A list of commonly used NSAIDs is given in the following table:

NSAID	Some common trade names
Acetaminofene	Tylenol
Cimicifuga	Artrol
Choline salicylate-Mg salicylate	Trilisate
Diclofenac	as Na salt: Apo-Diclo, Apo-Diclo SR, Arthrotec, Diclofenac Ect, Novo-Difenac, Novo-Difenac SR, Nu-Diclo, Taro-Diclofenac, Voltaren, Voltaren SR; as K salt: Voltaren Rapide
Diflunisal	Apo-Diflunisal, Dolobid, Novo-Diflunisal, Nu-Diflunisal
Etodolac	Ultradol
Fenoprofen calcium	Nalfon
Floctafenine	Idarac

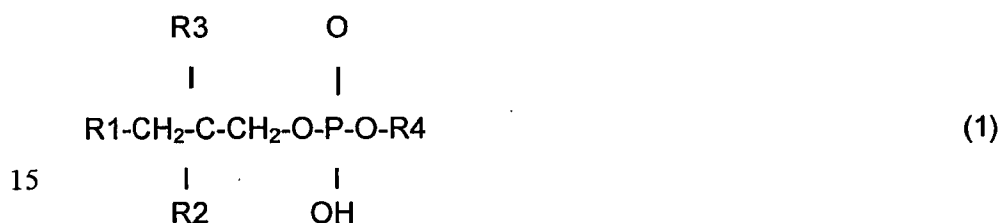
- 28 -

	Flurbiprofen	Ansaid, Apo-Flurbiprofen FC, Froben,
	Ibuprofen	Froben SR, Novo-Flurprofen, Nu-Flurbiprofen
5		Actiprofen, Advil, Advil Cold & Sinus, Amersol,
		Apo-Ibuprofen, Excedrin IB, Medipren, Motrin,
	Indomethacin	Motrin IB, Novo-Profen, Nuprin, Nu-Ibuprofen
		Apo-Indomethacin, Indocid, Indocid SR,
		Indolec, Novo-Methacin, Nu-Indo, Pro-Indo,
		Rhodacine
10	Ketoprofen	Apo-Keto, Apo-Keto-E, Novo-Keto,
		Novo-Keto-Ec, Nu-Ketoprofen,
		Nu-Ketoprofen-E, Orudis, Orudis E,
		Orudis SR, Oruvail, PMS-Ketoprofen, PMS-
		Ketoprofen-E, Rhodis, Rhodis-EC
	Ketorolac tromethamine	Acular, Toradol
15	Magnesium salicylate	Back-Ese-M, Doan's Backache Pills,
		Herbogesic
	Mefenamic acid	Ponstan
	Nabumetone	Relafen
20	Naproxen	Apo-Naproxen, Naprosyn, aprosyn-E, Naxen,
		Novo-Naprox, Nu-Naprox, PMS-Naproxen;
		or in the sodium form: Anaprox, Anaprox DS,
		Apo-Napro-Na, Naproxin-Na, Novo-Naprox
		Sodium, Synflex, Synflex DS
	Oxyphenbutazone	Oxybutazone
25	Phenylbutazone	Alka Phenyl, Alka Phenylbutazone, Apo-
		Phenylbutazone, Butazolidin, Novo-Butazone,
		Phenylone Plus
	Piroxicam	Apo-Piroxicam, Feldene, Kenral-Piroxicam,
30		Novo-Pirocam, Nu-Pirox, PMS-Piroxicam, Pro-
		Piroxicam, Rho-Piroxicam

- 29 -

	Salsalate	Disalcid
	Sodium salicylate	Apo-Sulin, Dodd's, Dodd's Extra-Strength, Sulindac, Clinoril, Novo-Sundac, Nu-Sulindac, Sulindac
5	Tenoxicam	Mobiflex
	Tiaprofenic acid	Albert Tiafen, Apo-Tiaprofenic, Surgam, Surgam SR
	Tolmetin sodium	Novo-Tolmetin, Tolectin

10 The term "**phospholipid**" has, for example, the formula



in which one of the radicals R1 and R2 represents hydrogen, hydroxy or C1-C4-alkyl, and the other radical represents a long fatty chain, especially an
 20 alkyl, alkenyl, alkoxy, akenyloxy or acyloxy, each having from 10 to 24 carbon atoms, or both radicals R1 and R2 represent a long fatty chain, especially an alkyl, alkenyl, alkoxy, alkenyloxy or acyloxy each having from 10 to 24 carbon atoms, R3 represents hydrogen or C1-C4-alkyl, and R4 represents hydrogen, optionally substituted C1-C7-alkyl or a carbohydrate
 25 radical having from 5 to 12 carbon atoms or, if both radicals R1 and R2 represent hydrogen or hydroxy, R4 represents a steroid radical, or is a salt thereof. The radicals R1, R2, R3, and R4 are typically selected so as to ensure that lipid bilayer membrane is in the fluid lamellar phase during practical application and is a good match to the drug of choice.

- 30 -

In a phospholipid of the formula 1, R1, R2 or R3 having the meaning C1-C4-alkyl is preferably methyl, but may also be ethyl, *n*-propyl, or *n*-butyl.

5 The terms alkyl, alkenyl, alkoxy, alkenyloxy or acyloxy have their usual meaning. The long fatty chains attached to a phospholipid can also be substituted in any of usual ways.

Alkyl R1 or R2 is preferably straight-chained with an even number of 10 to 24 carbon atoms, for example *n*-decyl, *n*-dodecyl (lauryl), *n*-tetradecyl (myristyl),
10 *n*-hexadecyl (cetyl), *n*-octadecyl (stearyl), *n*-eicosyl (arachinyl), *n*-docosyl (behenyl) or *n*-tetracosyl (lignoceryl). In this and all the related following definitions, the intermediate odd-numbered derivatives are useful, but are less preferred.

15 Alkenyl R1 and/or R2 is preferably straight-chained with an even number of 12 to 24 carbon atoms and a double bond, for example 9-*cis*-dodecenyl (lauroleynyl), 9-*cis*-tetradecenyl (myristoleynyl), 9-*cis*-hexadecenyl (palmitoleynyl), 9-*cis*-octadecenyl (petroselinyl), 6-*trans*-octadecenyl (petroselaidinyl), 9-*cis*-octadecenyl (oleyl), 9-*trans*-octadecenyl (elaidinyl), 9-*cis*-eicosenyl
20 (gadoleinyl), 9-*cis*-docosenyl (cetoleinyl) or 9-*cis*-tetracosenyl (nervonyl). In this and all the related following definitions, the other corresponding trans-derivatives are potentially useful as well but are less preferred.

Alkoxy R1 and/or R2 is preferably straight-chained with an even number of
25 10 to 24 carbon atoms, for example *n*-decyloxy, *n*-dodecyloxy (lauryloxy), *n*-tetradecyloxy (myristyloxy), *n*-hexadecyloxy (cetyloxy), *n*-octadecyloxy (stearyloxy), *n*-eicosyloxy (arachinyloxy), *n*-docosoyloxy (behenyloxy) or *n*-tetracosoyloxy (lignoceryloxy).

30 Alkenyloxy R1 and/or R2 is preferably straight-chained with an even number of 12 to 24 carbon atoms, for example 9-*cis*-dodecenyloxy (lauroleyloxy), 9-

- 31 -

cis-tetradecenyl (myristoleyl), 9-*cis*-hexadecenyl (palmitoleyl), 6-*cis*-octadecenyl (petroselinyl), 6-*trans*-octadecenyl (petroselaidyl), 9-*cis*-octadecenyl (oleyl), 9-*trans*-octadecenyl (elaidyl), and 9-*cis*-eicosenyl (gadoleyl), 9-*cis*-docosenyl (cetoleyl) or 9-*cis*-tetracosyl (nervonyl).

Acyloxy R1 and/or R2 is preferably straight-chained with an even number of 10 to 24 carbon atoms, for example alkanoyloxy or alkenoyloxy, preferably *n*-decanoyloxy, *n*-dodecanoyloxy (lauroyl), *n*-tetradecanoyloxy (myristoyl), *n*-hexadecanoyloxy (palmitoyl), *n*-octadecanoyloxy (stearoyl), *n*-eicosanoyloxy (arachinoyl), *n*-docosanoyloxy (behenoyl) and *n*-tetracosanoyloxy (lignoceroyl).

Alkenoyloxy R1, and/or R2 is preferably straight-chained with an even number of 10 to 20 carbon atoms, for example 9-*cis*-dodecenyl (lauroleoyl), 9-*cis*-tetradecenyl (myristoleoyl), 9-*cis*-hexadecenyl (palmitoleyl), 6-*cis*-octadecenyl (petroselinyl), 6-*trans*-octadecenyl (petroselaidyl), 9-*cis*-octadecenyl (oleyl), 9-*trans*-octadecenyl (elaidyl), and 9-*cis*-eicosenyl (gadoleyl), 9-*cis*-docosenyl (cetoleyl) and 9-*cis*-tetracosenyl (nervonyl).

Optionally substituted C1-C7-alkyl R4 is, for example, methyl, ethyl, isopropyl, *n*-propyl, isobutyl or *n*-butyl, which can be substituted by acidic groups, for example, carboxy or sulphy, by acidic and basic groups, for example, carboxy and amino, the amino group being in the α -position to the carboxy group, by free or etherified hydroxy groups, it being possible for two etherified hydroxy groups to be bonded to one another by a bivalent hydrocarbon radical, for example methylene, ethylene, ethylidene, 1,2-propylene or 2,2-propylene; or by halogen, for example chlorine or bromine,

by lower alkoxy-carbonyl, for example methoxy- or ethoxy-carbonyl, or by lower alkanesulphonyl, for example methanesulphonyl.

Substituted C1-C7-alkyl R4 is, for example, carboxy-lower alkyl, for example
5 carboxymethyl, 2-carboxyethyl or 3-carboxy-*n*-propyl, (omega-amino-omega-carboxy-lower alkyl, for example 2-amino-2-carboxyethyl or 3-amino-3-carboxy-*n*-propyl, hydroxy-lower alkyl, for example 2-hydroxyethyl or 2,3-dihydroxypropyl, lower alkoxy-lower alkyl, for example methoxy- or ethoxymethyl, 2-methoxyethyl or 3-methoxy-*n*-propyl, lower alkylenedioxy-lower
10 alkyl, for example 2,3-ethylenedioxypropyl or 2,3-(2,2-propylene)-dioxypropyl, or halo-lower alkyl, for example chloro- or bromo-methyl, 2-chloro- or 2-bromo-ethyl, 2- or 3-chloro- or 2- or 3-bromo-*n*-propyl.

Substituted C1-C7-alkyl R4 is preferably ethyl substituted by tri-lower
15 alkylammonium, for example trimethyl- or triethyl-ammonium, for example 2-trimethylammonium-ethyl or 2-ammonium-ethyl, or is, for example omega-amino-omega-carboxy-lower alkyl, for example 2-amino-2-carboxyethyl.

A carbohydrate radical R4 having from 5 to 12 carbon atoms is, for example,
20 a natural monosaccharide radical that is derived from a pentose or hexose present in the form of aldose or ketose. Detailed definitions of most relevant carbohydrate radicals (pentoses, hexoses, disaccharides, etc.) is given in the patent EP 0 475 160 by the same applicant.

25 A steroid radical R4 is, for example, a sterol radical that is esterified by the phosphatidyl group by way of the hydroxy group located in the 3-position of the steroid nucleus.

A sterol radical is, for example, the lanosterol, sitosterol, coprostanol,
30 cholestanol, glycocholic acid, ergosterol or stigmasterol radical, preferably the cholesterol radical.

If R4 represents a steroid radical, R1 and R2 are preferably hydroxy and R3 is hydrogen.

- 5 Phospholipids of the formula 1 can be in the form of free acids or in the form of salts. Salts are formed by reaction of the free acid of the formula II with a base, for example a dilute, aqueous solution of alkali metal hydroxide, for example lithium, sodium or potassium hydroxide, magnesium or calcium hydroxide, a dilute aqueous ammonia solution or an aqueous solution of an
- 10 amine, for example a mono-, di- or tri-lower alkylamine, for example ethyl-, diethyl- or triethyl-amine, 2-hydroxyethyl-tri-C1-C4-alkyl-amine, for example choline, and a basic amino acid, for example lysine or arginine.

- A phospholipid of the formula 1 has especially two acyloxy radicals R1 and
- 15 R2, for example alkanoyloxy or alkenoyloxy, for example lauroyloxy, myristoyloxy, palmitoyloxy, stearoyloxy, arachinoyloxy, oleoyloxy, linoyloxy or linoleoyloxy, and is, for example, natural lecithin (R3 = hydrogen, R4 = 2-trimethylammonium ethyl) or cephalin (R3 = hydrogen, R4 = 2-ammonium ethyl) having different acyloxy radicals R1 and R2, for example egg lecithin or
- 20 egg cephalin or lecithin or cephalin from soy beans, synthetic lecithin (= phosphatidylcholine) or cephalin (= phosphatidylethanolamine) having different or identical acyloxy radicals R1 and R2, for example 1-palmitoyl-2-oleoyl phosphatidylcholine or phosphatidylethanolamine or dipalmitoyl, distearoyl, diarachinoyl, dioleoyl, dilinoyl or dilinoleoyl phosphatidylcholine or
- 25 phosphatidylethanolamine, natural phosphatidyl serine (R3 = hydrogen, R4 = 2-amino-2-carboxyethyl) having different acyloxy radicals R1 and R2, for example phosphatidyl serine from bovine brain, synthetic phosphatidylserine having different or identical acyloxy radicals R1 and R2, for example dioleoyl-, dimyristoyl- or dipalmitoyl-phosphatidyl serine, or natural phosphatidic acid
- 30 (R3 and R4 = hydrogen) having different acyloxy radicals R1 and R2.

- 34 -

A phospholipid of the formula 1 is also a phospholipid in which R1 and R2 represent two identical alkoxy radicals, for example *n*-tetradecyloxy or *n*-hexadecyloxy (synthetic ditetradecyl or dihexadecyl phosphatidylcholine or phosphatidylethanolamine), R1 represents alkenyl and R2 represents acyloxy, for example myristoyloxy or palmitoyloxy (plasmalogen, R3 = hydrogen, R4 = 2-trimethylammonium ethyl), R1 represents acyloxy and R2 represents hydroxy (natural or synthetic lysophosphatidylcholine or lysophosphatidylethanolamine, for example 1-myristoyl- or 1-palmitoyl-lyso-
10 phosphatidylcholine or - phosphatidylethanolamine; natural or synthetic lysophosphatidyl serine, R3 = hydrogen, R4 = 2-amino-2-carboxyethyl, for example lysophosphatidyl serine from bovine brain or 1-myristoyl- or 1-palmitoyl-lysophosphatidyl serine, synthetic lysophosphatidyl glycerine, R3 = hydrogen, R4 = CH₂OH-CHOH-CH₂-, natural or synthetic lysophosphatidic
15 acid, R3 = hydrogen, R4 = hydrogen, for example egg lysophosphatidic acid or 1-lauroyl-, 1-myristoyl- or 1-palmitoyl-lysophosphatidic acid).

A lipid that is analogous to abovementioned phospholipid and can replace the latter is, for example, a lysophosphatidylcholine analogue, for example 1-
20 lauroyl-1,3-propanediol-3- phosphoryl choline, a monoglyceride, for example monoolein or monomyristin, a cerebroside, a ganglioside, or a glyceride that does not contain a free or esterified phosphoryl or phosphonyl group in the 3-position, for example a diacylglyceride or 1-alkenyl-1-hydroxy-2-acylglyceride, having the mentioned acyl or alkenyl groups in which the 3-
25 hydroxy group is etherified by one of the mentioned carbohydrate radicals, for example a galactosyl radical, for example monogalactosyl glycerine.

The lipids and certain surfactants mentioned hereinbefore and hereinafter having a chiral carbon atom can be present both in the form of racemic

- 35 -

mixtures and in the form of optically pure enantiomers in the pharmaceutical compositions that can be prepared and used according to the invention.

The term "**sterol radical**" means, for example, the lanosterol, sitosterol,
5 coprostanol, cholestanol, glycocholic acid, ergosterol or stigmasterol radical, is preferably the cholesterol radical, but can also be any other sterol radical known in the art.

The term "**surfactant**" also has its usual meaning. A long list of relevant
10 surfactants and surfactant related definitions is given in EP 0 475 160 and USP 6 165 500 which are herewith explicitly included by reference and in appropriate surfactant or pharmaceutical Handbooks, such as *Handbook of Industrial Surfactants* or US Pharmacopoeia, Pharm. Eu., etc.. The following list therefore only offers a selection, which is by no means complete or
15 exclusive, of several surfactant classes that are particularly common or useful in conjunction with present patent application. This includes ionised long-chain fatty acids or long chain fatty alcohols, long chain fatty ammonium salts, such as alkyl- or alkenoyl-trimethyl-, -dimethyl- and -methyl-ammonium salts, alkyl- or alkenoyl-sulphate salts, long fatty chain dimethyl-aminoxides,
20 such as alkyl- or alkenoyl-dimethyl-aminoxides, long fatty chain, for example alkanoyl, dimethyl-aminoxides and especially dodecyl dimethyl-aminoxide, long fatty chain, for example alkyl-N-methylglucamides and alkanoyl-N-methylglucamides, such as MEGA-8, MEGA-9 and MEGA-10, N-long fatty chain-N,N-dimethylglycines, for example N-alkyl-N,N-dimethylglycines, 3-
25 (long fatty chain-dimethylammonio)-alkanesulphonates, for example 3-(acyldimethylammonio)-alkanesulphonates, long fatty chain derivatives of sulphosuccinate salts, such as bis(2-ethylalkyl) sulphosuccinate salts, long fatty chain-sulphobetaines, for example acyl-sulphobetaines, long fatty chain betaines, such as EMPIGEN BB or ZWITTERGENT-3-16, -3-14, -3-12, -3-10,
30 or -3-8, or polyethylen-glycol-acylphenyl ethers, especially nonaethylen-

- 36 -

glycol-octylphenyl ether, polyethylene-long fatty chain-ethers, especially polyethylene-acyl ethers, such as nonaethylen-decyl ether, nonaethylen-dodecyl ether or octaethylene-dodecyl ether, polyethyleneglycol-isoacyl ethers, such as octaethyleneglycol-isotridecyl ether, polyethyleneglycol-

5 sorbitane-long fatty chain esters, for example polyethyleneglycol-sorbitane-acyl esters and especially polyethylenglykol-monolaurate (e.g. Tween 20), polyethylenglykol-sorbitan-monooleate (e.g. Tween 80), polyethylenglykol-sorbitan-monolauroleylate, polyethylenglykol-sorbitan-monopetroselinate, polyethylenglykol-sorbitan-monoelaidate, polyethylenglykol-sorbitan-

10 myristoleylate, polyethylenglykol-sorbitan-palmitoleinylate, polyethylenglykol-sorbitan-petroselinyrate, polyhydroxyethylene-long fatty chain ethers, for example polyhydroxyethylene-acyl ethers, such as polyhydroxyethylene-lauryl ethers, polyhydroxyethylene-myristoyl ethers, polyhydroxyethylene-cetylstearyl, polyhydroxyethylene-palmityl ethers, polyhydroxyethylene-oleoyl

15 ethers, polyhydroxyethylene-palmitoleoyl ethers, polyhydroxyethylene-linoleyl, polyhydroxyethylen-4, or 6, or 8, or 10, or 12-lauryl, miristoyl, palmitoyl, palmitoleyl, oleoyl or linoeyl ethers (Brij series), or in the corresponding esters, polyhydroxyethylen-laurate, -myristate, -palmitate, -stearate or -oleate, especially polyhydroxyethylen-8-stearate (Myrj 45) and

20 polyhydroxyethylen-8-oleate, polyethoxylated castor oil 40 (Cremophor EL), sorbitane-mono long fatty chain, for example alkylate (Arlacel or Span series), especially as sorbitane-monolaurate (Arlacel 20, Span 20) or monooleate, long fatty chain, for example acyl-N-methylglucamides, alkanoyl-N-methylglucamides, especially decanoyl-N-methylglucamide,

25 dodecanoyl-N-methylglucamide or octadecanoyl-N-methylglucamide, long fatty chain sulphates, for example alkyl-sulphates, alkyl sulphate salts, such as lauryl-sulphate (SDS), oleoyl-sulphate; long fatty chain thioglucosides, such as alkylthioglucosides and especially heptyl-, octyl-, nonyl and decyl-beta-D-thioglucopyranoside; long fatty chain derivatives of various

30 carbohydrates, such as pentoses, hexoses and disaccharides, especially

alkyl-glucosides and maltosides, such as hexyl-, heptyl-, octyl-, nonyl- and decyl-beta-D-glucopyranoside or D-maltopyranoside; further a salt, especially a sodium salt, of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, a fatty acid salt, especially oleate, elaidate, linoleate, laurate, or myristate, most often in sodium form, lysophospholipids, *n*-octadecylene-glycerophosphatidic acid, octadecylene-phosphorylglycerol, octadecylene-phosphorylserine or phosphatidylcholine, *n*-long fatty chain-glycero-phosphatidic acids, such as *n*-acyl-glycero-phosphatidic acids, especially lauryl glycero-phosphatidic acids, oleoyl-glycero-phosphatidic acid, *n*-long fatty chain-phosphorylglycerol, such as *n*-acyl-phosphorylglycerol, especially lauryl-, myristoyl-, oleoyl- or palmitoeloyl-phosphorylglycerol, *n*-long fatty chain-phosphorylserine, such as *n*-acyl-phosphorylserine, especially lauryl-, myristoyl-, oleoyl- or palmitoeloyl-phosphorylserine, *n*-tetradecyl-glycero-phosphatidic acid, *n*-tetradecyl-phosphorylglycerol, *n*-tetradecyl-phosphorylserine, the corresponding elaidoyl-, vaccenyl-lysophospholipids, the corresponding short-chain phospholipids, as well as all surface active and thus membrane destabilising polypeptides. Surfactant chains are typically chosen to be in a fluid state or at least to be compatible with the maintenance of fluid-chain state in carrier aggregates.

20

The term "**surfactant like phospholipid**" means a phospholipid with solubility, and other relevant properties, similar to those of the corresponding surfactants mentioned in this application, especially in the Claims 104 and 105. A non-ionic surfactant like phospholipid therefore should have water solubility, and ideally also water diffusion / exchange rates, etc., similar to those of a relevant non-ionic surfactant.

25

The lipids and certain surfactants mentioned hereinbefore and hereinafter having a chiral carbon atom can be present both in the form of racemic

mixtures and in the form of optically pure enantiomers in the pharmaceutical compositions that can be prepared and used according to the invention.

Description of Figures

5

Figure 1: Shape deformation: Schematic representation of aggregate shape deformation during pore crossing.

10

Figure 2: Energy cost deformation: Energy level associated with different states of aggregate deformation that result from an enforced aggregate passing through a narrow pore in a semi-permeable barrier.

15

Figure 3: Deformation & Penetrability: Penetrability of a semi-permeable porous barrier to the suspension of vesicles smaller the average pore diameter in the barrier as a function of transbarrier pressure which drives the suspension through the barrier.

20

Figure 4: Molecular redistribution in an aggregate-enshrining lipid bilayer during aggregate deformation and pore crossing, which lowers the activation energy for transbarrier transport.

25

Figure 5: Schematic illustration of the role played by membrane destabilising component(s) on lipid bilayer adaptability. The effect of relative concentration of the second membrane destabilising component is shown in inset. Bilayer vesicle adaptability as a function of absolute concentration of membrane destabilising components (surfactants; $MDC_1 + MDC_2$) and of the relative concentration of such components in the mixed, three component bilayers based on a lipid (MFC), as the membrane forming component. The presence of second membrane destabilising component can increase bilayer adaptability

30

disproportionally, arguably by increasing lipid bilayer deformability and permeability. This is presumably due to MDC_1 - MDC_2 coupling/interaction, which here is proportional to the parameter m ; although not represented, MFC - MDC_1 or MFC - MDC_2 coupling/interaction can be similarly important.

- 5 Absolute adaptability values and precise curve form depend on specific choice of model parameters and therefore can be different from the shown ones.

Figure 6: Effect of second membrane destabilising amphipat (SDS; MDC_2) in the mixed phospholipid (SPC, MFS) Tween 80 (first membrane destabilising amphipat, MDC_1) bilayers with increasing relative concentration of the latter on Barrier Transport Resistance, measured with a simple experimental method (SEM). The curves are drawn merely to guide the eye. The effect of changing molar ratio of the second (Tween80=) and the third (surfactant; SDS) amphipatic system component, relatively to the first amphipatic system component (phospholipid; SPC), on the resistance of resulting mixed lipid suspension to the filtration through a barrier with 0.2 micrometer pore-diameter (left panel) is shown. The starting and final vesicle diameter was significantly greater than the average pore diameter.

Figure 7: Pressure dependence barrier penetrability to three different suspensions of mixed bilayer vesicles, pushed through narrow pores, as a function of the second surfactant concentration. Exemplified is the effect of a charged biosurfactant, sodium cholate, in mixtures with another surfactant (Tween 80) containing phospholipid bilayers on the ability of the resulting lipid vesicle suspensions to penetrate through a semipermeable barrier under influence of transbarrier hydrostatic pressure. Pressure dependence barrier penetrability to three different

- 40 -

suspensions of mixed bilayer vesicles, pushed through narrow pores, as a function of the second surfactant concentration.

Figure 8: illustrates penetrability of the suspensions prepared as described in examples 143 (●) and 144 (○). The curves were calculated within the framework of Maxwell's energy distribution model, by using formula (*).

Figure 9: Penetration curves for different SPC/KT mixtures: $\Delta=2.5/1$ SPC/KT, ♦ 3/1 SPC/KT, ▽ 4/1 SPC/KT, □ SPC/Tween 1/1 Transfersomes[®] as a Reference suspension. The curves were calculated within the framework of the data fitting model described herein before, by using eq. (*).

Figure 10: Penetration curves for SPC/KT 3/1 mole/mole formulation without (○) and with (●) 10 rel-mol% of Tween 80. Reference Tween-Transfersomes[®] *. The curves were fitted to the data using eq. (*).

Figure 11: Area under the curve (AUC), which reflects the cumulative delivery of the drug, calculated from the pharmacokinetic results measured with different ketoprofen (KT) formulations tested in pigs (n = 4).

Detailed description of the Invention

The invention describes suspensions of complex ESAs with at least three amphipatic components, one of which is membrane forming and at least two of which are membrane destabilising, which can be suspended in a suitable, e.g. pharmaceutically acceptable, polar liquid medium and loaded with at least one biologically active compound, which can correspond to one of the amphipats. An essential characteristic of such, relatively large, aggregates is the ability to penetrate pores in semi-permeable barriers even when the pore radius is significantly, i.e. at least 25% and often is more than 40% or even

- 41 -

better more than 50% and most preferably is more than 70% smaller than the average aggregate radius before barrier crossing. Another important characteristic of aggregates introduced in this document is the relatively low concentration of one of the two membrane destabilising components, which
5 is below the concentration needed to achieve high aggregate shape deformability when this component is used for the purpose on its own. High aggregate deformability is a prerequisite for reaching practically useful-i.e. sufficiently high-suspension flux through a barrier, such that approaches in order of magnitude the flux of suspending medium. The other necessary
10 condition is sufficient aggregate stability, which ensures that the average aggregate radius after barrier crossing is still at least 40%, more often is at least 50% and most typically is at least 100% larger than the pore radius. High deformability and sufficient stability of aggregates that can cross semipermeable barriers are sub-summarised in the term aggregate
15 adaptability, which is parameterised as a_a . Highly adaptable complex aggregates excel in their ability to transport active ingredients through semipermeable barriers, such as mammalian skin.

The present invention specially relates to the selection of one membrane
20 destabilizing amphipatic component of the system such that can boost the deformability of mixed aggregates supported by judicious choice of the other system components to the effect of improving barrier penetration by such aggregates. The invention also teaches how to select the right total amphipat concentration of and, in case, amphipat
25 ionisation in mixed aggregate suspensions. The invention further relates to the preparation and application of resulting suspensions in pharmaceutical formulations, with a focus on epicutaneous application on, or less frequently in, the warm blood creatures.

- 42 -

We discovered unexpectedly that incorporation of an additional, suitable amphipatic membrane destabilising component (MDC_2) in aforementioned bi-component ($MFC + MDC_1$) aggregates can increase the resulting three-component ($MFC + MDC_1 + MDC_2$) aggregate adaptability $a_a(MFC + MDC_1 + MDC_2) > a_a(MFC + MDC_1)$ and thus augments the shape deformability of resulting aggregates. This lowers the pressure p^* needed to drive substantial suspension flux through a barrier: $p^*(MFC + MDC_1 + MDC_2) < p^*(MFC + MDC_1)$. The capability of said at least three-component aggregates to move through a semi-permeable barrier is therefore increased. This finding is surprising taken that the droplets covered by a bi-component bilayer membrane already have a rather high barrier crossing ability compared to the droplets enshrined by a simple lipid bilayer: $a_a(MFC + MDC_1) \gg a_a(MFC)$.

Apparently, the third aggregate component, which acts as a second membrane destabilising component, can increase or support transport-permitting aggregate adaptability beyond normal expectation: $a_a(MFC + MDC_1 + MDC_2) > a_a(MFC + MDC_1)$ and $a_a(MFC + MDC_1 + MDC_2) > a_a(MFC + MDC_2)$. This is illustrated in inset to figure 5.

The three-component bilayer membrane comprising a lipid (MFC), a suitable first surfactant / amphipatic drug (MDC_1) and a suitable second surfactant / amphipatic drug (MDC_2) may also require a lower driving pressure to achieve transbarrier transport: $p^*(MFC + MDC_1 + MDC_2) < p^*(MFC + MDC_2)$. Additionally or alternatively, a lower total amount of bilayer destabilising second amphipat may suffice for obtaining sufficiently adaptable aggregates, such that can cross a semipermeable barrier. The role of both membrane destabilising compounds is potentially, but not necessarily quantitatively, interchangeable (cf. figure 5).

Specifically, we found that relative concentration of said third component, which acts as membrane destabilising amphipat in the at least quaternary suspension (liquid suspending medium + MFC + MDC_1 + MDC_2 preferably water+lipid+drug+surfactant) containing aggregates with a high adaptability, can be kept below the necessary MDC_2 , preferably the surfactant, concentration in a ternary suspension (liquid suspending medium + MFC + MDC_2 preferably water+lipid+surfactant) containing aggregates of similar adaptability: $a_a(MFC + MDC_1 + MDC_2)$

10 $\approx a_a(MFC + MDC_2)$ and $[MDC_2]_{\text{three-component}} < [MDC_2]_{\text{bi-component}}$ or $a_a(MFC + MDC_1 + MDC_2) \approx a_a(MFC + MDC_1)$ and $[MDC_1]_{\text{three-component}} < [MDC_1]_{\text{bi-component}}$, values in square brackets denoting molar membrane component concentrations. Practical Examples provide several illustrations for this. In our opinion, this phenomenon reflects a synergy between the action of

15 two bilayer components, e.g. between both membrane destabilising constituents (preferably amphipatic drug(s), surfactant(s); MDC_1 , MDC_2). The dependence of adaptability curve on the magnitude of coupling parameter m , documented in inset to figure 5, supports such notion. We furthermore suggest that the interacting two membrane destabilizing

20 components together make said three-component lipid bilayers more permeable and/or more flexible than the two-component bilayer membranes in which one of these MDC is lacking. This means that: $a_a([MFC] + [MDC_1] + [MDC_2]) > a_a([MFC] + [MDC_1])$ and $a_a([MFC] + [MDC_1] + [MDC_2]) > a_a([MFC] + [MDC_2])$, similar concentration

25 symbols meaning identical membrane component concentration. The corresponding p^* values typically exhibit the inverse behaviour of a_a values.

Preferably, the aggregate adaptability fulfils the condition $a_a([MFC] + [MDC_1] + [MDC_2]) > a_a([MFC] + [MDC_1])$ and/or $a_a([MFC] + [MDC_1] + [MDC_2]) > a_a([MFC] + [MDC_2])$

30

- 44 -

$[MDC_2] > a_a([MFC] + [MDC_2])$, wherein the combined molar concentration of both membrane destabilizing compounds $[MDC_1] + [MDC_2]$ in aggregates comprising three amphipats ($MFC + MDC_1 + MDC_2$) is equal or less than the molar concentration of $[MDC_1]$ in the aggregates that
 5 comprise only two amphipats ($MFC + MDC_1$) and/or is less than the molar concentration of $[MDC_2]$ in the aggregates comprising only two amphipats ($MFC + MDC_2$), at the same molar concentration of MFC, or the aggregate adaptability fulfils the condition $a_a([MFC] + [MDC_1] + [MDC_2]) \approx a_a([MFC] + [MDC_1])$ and/or $a_a([MFC] + [MDC_1] + [MDC_2]) \approx$
 10 $a_a([MFC] + [MDC_2])$, wherein the combined molar concentration $[MDC_1] + [MDC_2]$ in aggregates comprising three amphipats ($MFC + MDC_1 + MDC_2$) is less than the molar concentration of $[MDC_1]$ in the aggregates comprising only two amphipats ($MFC + MDC_1$) and/or is less than the molar concentration of $[MDC_2]$ in the aggregates comprising only two
 15 amphipats ($MFC + MDC_2$), at the same molar concentration of MFC. The corresponding p^* values typically exhibit the inverse behaviour of a_a values.

Therefore, a second membrane destabilizing compound can be used to
 20 form aggregates comprising three amphipates ($MFC + 2 \text{ different MDCs}$) and thus achieve aggregate adaptability a_a which is higher than that of an aggregate comprising only two amphipats ($MFC + MDC$). Accordingly MDC_1 can be used to increase the adaptability a_a of an aggregate comprising MFC and MDC_2 , and MDC_2 can be used to increase the
 25 adaptability a_a of an aggregate comprising MFC and MDC_1 by forming an aggregate comprising three amphipats ($MFC + 2 \text{ different MDCs}$). Likewise the second membrane destabilizing compound can be used to decrease the amount of the first membrane destabilizing compound which would be necessary to achieve a certain adaptability a_a when used
 30 alone in an aggregate comprising two amphipats. Accordingly MDC_1 can

- 45 -

be used to form an aggregate comprising three amphipats ($MFC + MDC_1$ and MDC_2) to lessen the amount of MDC_2 necessary when used alone in an aggregate comprising MFC and MDC_2 to achieve a certain adaptability a_a and/or MDC_2 can be used to form an aggregate

5 comprising three amphipats ($MFC + MDC_1$ and MDC_2) to lessen the amount of MDC_1 necessary when used alone in an aggregate comprising MFC and MDC_1 to achieve a certain adaptability. Preferably the second membrane destabilizing compound MDC_1 or MDC_2 is used to form an aggregate comprising three amphipats ($MFC + MDC_1 + MDC_2$) whereby

10 the total molar amount of destabilizing compound necessary to achieve a certain adaptability of an aggregate comprising two amphipats, one membrane forming compound and the respective other membrane destabilizing compound ($MFC + MDC_1$) or ($MFC + MDC_2$), is reduced, so $[MDC_1] + [MDC_2]$ in amphipats comprising $[MFC] + [MDC_1] + [MDC_2]$ is

15 less than $[MDC_1]$ in amphipats comprising $[MFC] + [MDC_1]$ and/or $[MDC_2]$ in amphipats comprising $[MFC] + [MDC_2]$.

We note that the characteristics listed in previous paragraph favourably affect the transport of said pluri-component mixed lipid vesicles through

20 the skin. Simultaneous presence of at least two bilayer destabilising amphipats in aggregate suspension based on the lipid that forms stable bilayers is therefore beneficial for application of corresponding pharmaceutical formulations on semi-permeable barriers, such as the skin.

25

We thus unveil a fairly general, previously unknown phenomenon with great practical and commercial potential. An example is the transport of drugs across various biological barriers mediated by the three-

30 destabilising amphipats) in said at least quaternary mixture. The

- 46 -

requirement for this is the capability of complex aggregates to cross pores with a radius at least 25% smaller than the average aggregate radius before passage through the pores. The pores can also be part of the pathway through the skin, which makes said at least quaternary
5 mixtures suitable for transdermal drug delivery. Quaternary mixtures containing at least one polar, but poorly soluble lipid (which on its own forms extended aggregates) and at least two relatively highly soluble amphipats (surfactants / drugs, which tend to destabilise the aforementioned lipid bilayer), consequently can improve drug transport
10 into the body of warm blood creatures.

Most drugs are amphipatic. Many such molecules, especially in the ionised form, are also edge active and are thus attracted to the hydrophilic-hydrophobic boundaries. Some drugs may self-aggregate or at least tend to
15 adsorb to an air-water or lipid-water interface; this is mainly due to hydrophobic, ionic, or H-bond interactions between drugs and lipid (aggregates), which can lead to the creation of weak drug-lipid associates. The solubility and/or amphipaty of such associates typically are greater than that of the involved lipid or drug alone. This is the reason why amphipatic
20 drugs under certain conditions can destabilise or even permeate and solubilise lipid bilayer membranes. Such drugs then act as membrane destabilising components (*MDC*) in the sense of the present invention, but this is not necessarily the case under all conditions. Typically, sufficiently high drug solubility and sufficiently high drug partition coefficient in or binding
25 constant to a bilayer membrane are both required for the effect. The specific, suitable value for these two parameters depends on choice of other system characteristics (pH, salt and its concentration, lipid concentration, water activity, etc.). The rule of thumb is that the highest membrane-concentration of the most water-soluble drug form normally will work best, stability
30 considerations permitting. These conditions are also fulfilled for the drugs

with a solubilising capability used for preparing mini-droplets according to EP 0 475 160.

To solve the above mentioned problems, this invention describes
5 preparations based on a combination of at least one first, at least one second, and at least one third amphipatic component suspended in a suitable liquid medium in the form of corresponding mixed amphipat aggregates with one or a few bilayer-like, mixed amphipat coating(s), in which the combination of all three said components form extended surfaces in contact
10 with said liquid medium that are at least 50% more extended, on the average, than the typical surface of the aggregates comprising the said at least one second and at least one third amphipatic component alone and the adaptability of extended surface aggregates comprising all three said amphipatic components to ambient stress exceeds by at least 20% or by at
15 least twice the standard deviation of a typical measurement, whichever is smaller, the adaptability of the aggregates with extended surface that comprises the at least one first and the at least one second amphipatic component used at the corresponding concentrations or the adaptability of the extended surface comprising the at least one first and the at least one
20 third amphipatic component at corresponding concentrations, whichever is smaller, for the application, administration or transport of an active ingredient, which can be one of the three amphipatic components, especially for biological, medical, immunological, or cosmetic purposes, into and through the pores in semi-permeable barriers or other constrictions, such as through
25 the skin of warm blood creatures or the like.

In an alternative definition of the described problems solution, a combination of at least one first, at least one second, and at least one third amphipatic component suspended in a suitable liquid medium in the form of mixed
30 amphipat aggregates with one or a few bilayer-like, mixed amphipat coating(s), and thus with an extended surface, is used, in which the said at

- 48 -

least one first amphipatic component, on the one hand, and said at least one second and one third amphipatic components, on the other hand, have at least 2-times different solubilities in said liquid medium, and said at least one first substance has a tendency to self aggregate and is at least 10-times less
5 soluble in said liquid medium than said at least one second and said one third substance, allowing the first to form extended surfaces; furthermore, said at least one second substance is at least 10-times more soluble in said liquid medium and, on its own, tends to form or supports the formation of surfaces that are at least 2-times less extended than the surfaces containing
10 the at least one first substance alone and said at least one third substance is also at least 10-times more soluble in said liquid medium than the first substance and may, but needs not, form self-aggregates with aggregation numbers at least 10-times smaller than that of self-aggregates of said first substance; and said extended surfaces comprising said at least one first, at
15 least one second and at last one third substance, in equilibrium, have at least 50% greater extended surfaces than the surfaces formed by the at least one second or one third substance alone and/or both together, and preferably the aggregates with an extended surface comprising all three said amphipatic components have adaptability to ambient stress that exceeds by at least 20%
20 or by at least twice the standard deviation of a typical measurement, whichever is smaller, provided that the adaptability of the extended surface comprising the at least one first and the at least one second amphipatic component used at the corresponding concentrations or the adaptability of the extended surface comprising the at least one first and the at least one
25 third amphipatic component at the corresponding concentrations, whichever is smaller, all of which serves the purpose of application, administration or transport of at least one active ingredient, which can be amongst said three substances, especially for medicinal or biological purposes, into and through barriers and constrictions, such as the skin of warm blood creatures or the
30 like.

- 49 -

A favourable problem solution relies on use of said extended surfaces in the form of membrane surfaces.

- 5 Suitable combinations also fulfil the requirements as stated in previous paragraphs, simultaneously ensuring that the said at least one second substance increases the flexibility of extended surfaces comprising said at least one first, at least one second, and at least one third substance in comparison with the surfaces formed merely by an at least one first
10 substance or else with the surfaces formed by at least one first and at least one third substance.

- Further suitable combinations fulfil the requirements by ensuring that the said at least one second and one third substance together increase the
15 permeability of extended surfaces containing the said at least one first, at least one second, and at least one third substance, in comparison with the surfaces formed merely by the at least one first substance or else with the surfaces formed by at least one first and at least one third substance.

- 20 Combinations, which also fulfil said requirements contain said at least one second substance such that increases the ability to tolerate high curvature, as assessed by relative stability of said aggregates with an extended surface comprising said one first, said one second and said one third substance against enforced higher curvature during passing through a constriction with
25 maximum diameter at least 1.4 times smaller than the average diameter of an extended surface formed by an at least one first substance alone.

- When expressed in terms of relative solubilities of different components, combinations as taught by this application preferably comprise at least one
30 first substance and the at least one second substance that differ in solubility in the suspending medium on the average at least 10-fold. Preferably and/or

alternatively, the at least one second substance and the at least one third substance differ in solubility in the suspending medium on the average at least 2-fold.

- 5 It is furthermore recommendable to use combinations, as described in previous paragraphs, characterised by the fact that the concentration of said at least one second substance used in the combination with said one first and said one third substance is below 80% of the concentration that would be needed to render the aggregates comprising only said one first and said one
- 10 second substance as adaptable to ambient stress as the selected combination of all at least three substances. In preferred combinations according to the description in penultimate paragraph, the concentration of said at least one second substance amounts to at least 0.1% of the relative stated concentration. In further preferred combination, the concentration of
- 15 said at least one second substance amounts to 1 – 80% of the relative stated concentration.

- It is also possible to define the combination suitable for solving the problems described in this application by selecting relative concentration of said at
- 20 least one third substance, used in combination with said one first and said one second substance, to be above 0.1% of maximum possible concentration of the said at least one third substance in the system, a) as defined in terms of the solubility of said third substance in the system or in said at least three-component aggregates, or else b) as determined by the
- 25 negative action of said at least one third substance on the stability of said at least three-component aggregates. This means that more than 0.1% of saturating concentration of said third substance in the at least three component aggregates is preferably used or else , that the 0.1% limit pertains to maximum possible concentration of said third substance which

results in at least three-component aggregates to fail to fulfil the necessary aggregate stability criterion defined previously in the text.

Furthermore, it is possible to define a suitable combination by requesting
5 relative concentration of said at least one third substance used in
combination with said one first and with said one second substance to be
between 1% and 99%, more favourably to be between 10% and 95%, and
most preferably to be between 25% and 90% of maximum possible
concentration of said at least one third substance, a) as defined in terms of
10 the solubility of said third substance in the system or in said at least three-
component aggregates, or else b) as determined by the detrimental effect of
said at least one third substance on the stability of said at least three-
component aggregates, the qualitative meaning of these definitions being
described in previous paragraph.

15 Problem solving amphipat combination preferably contains between
0.01 weight-% and 50 weight-% dry mass as total mass of all at least three
amphipatic substances, which together form highly adaptable aggregates
with an extended surface. In more preferred formulations, this mass is
20 selected to be between 0.1 weight-% and 40 weight-%, even more preferably
between 0.5 weight-% and 30 weight-% and most preferably between 1
weight-% and 15 weight-%.

Amphipat combinations designed according to this application form extended
25 surfaces with a high adaptability, containing said at least three substances,
preferably with an average surface curvature corresponding to an average
radius between 15 nm and 5000 nm. A particularly favoured choice are the
systems with extended highly adaptable surfaces, which contain said at least
three substances, with an average curvature corresponding to an average

- 52 -

radius between 30 nm and 1000 nm, more preferably between 40 nm and 300 nm and most preferably between 50 nm and 150 nm.

Electrolyte composition and concentration affects the desirable properties of said amphipat combinations. It is therefore preferred to select these characteristics of the electrolyte in which the extended surfaces with at least one first, at least one second, and at least one third substance are suspended, and which comprises mono and/or oligovalent ions, so as to attain ionic strength between $I = 0.001$ and $I = 1$. A more preferred choice yields ionic strength between $I = 0.02$ and $I = 0.5$ which even more preferably is selected to be between $I = 0.1$ and $I = 0.3$.

Proton concentration in the selected electrolyte is an important parameter in case of ionizable systems. pH value of the suspending electrolyte therefore preferably should be chosen: a) in the vicinity of the logarithm of the apparent ionisation constant (pK_a) of said at least one second substance, if the latter is mono-ionizable, or in the vicinity of such pK_a value that maximises the solubility of said at least one second substance, if the latter has several ionizable groups, or else b) in the vicinity of pH optimum for the most rapidly decaying or the otherwise most sensitive amongst the said at least three substances, if the said at least one second substance is not ionizable. More specifically, the pH value of the polar medium in which the extended surfaces comprising at least one first, at least one second, and at least one third substance are suspended should be between $pH = pK_a - 3$ and $pH = pK_a + 3$, the final pH selection being also affected by said stability considerations. When a narrower choice is desirable, fixing electrolyte a) between $pH = pK_a - 1.5$ and $pH = pK_a + 2$, if said at least one third substance is more soluble at high pH , and b) between $pH = pK_a - 2$ and $pH = pK_a + 1.5$, if said at least one third substance is more soluble at low pH , is recommendable, the final pH selection again being subject to stability considerations.

- 53 -

A preferred solution to outlined problems is the use of said combinations characterised in that the at least one first substance, which is less soluble in the liquid medium and/or is the surface-building substance in the system, is a
5 lipid, preferably a phospholipid (e.g. as described herein before in the definition section), in that the at least one second substance, which is more soluble in the liquid medium and/or increases the tolerable surface curvature or adaptability of said extended surface, is a membrane destabilising amphipat and typically a surfactant, and in that said at least one third
10 substance is either a biologically active amphipatic ingredient, which has a capability of its own to increase the tolerable surface curvature or adaptability of said extended surface, or else is a different surfactant different from the said at least second substance. The second and third substance may be interchanged.

15
Some preferred amphipat combinations that can conveniently be used to solved the outlined problems are favourably arranged in the form of minute fluid droplets suspended or dispersed in a liquid, and surrounded by a coating of one or several layers of the at least one first substance, which is
20 capable of self-aggregation, and of at least one second substance and of at least one third substance, which are both amphipatic, provided that a) the former substance and the latter two substances differ in solubility in a suitable liquid suspending medium at least 10-fold, or else provided that b) the average radius of homo-aggregates of the more soluble amongst the at
25 least one second and third substance or of hetero-aggregates of the at least one first, the at least one second and the at least one third substance is smaller than the average radius of homo-aggregates of said at least one first substance, which is the least soluble amongst the three.

- 54 -

A preferred and practically very useful choice for the at least one first substance, as defined herein, is a polar or a non-polar, surface-forming lipid. This lipid is most often capable of forming bilayer membranes and preferably forms bilayers on its own. When looked upon from the solubility point of view, such surface-forming lipid can be dissolved in the liquid suspending medium e.g. suspension supporting polar medium preferably in a concentration range between 10^{-12} M and 10^{-7} M.

For biological applications it is commendable to select the at least one first substance forming extended surfaces as described in this document from the group of lipids, lipoids, from a biological source, corresponding synthetic lipids and biochemical or chemical modifications, i.e. derivatives, thereof.

Particularly preferred and attractive in the sense of previous paragraph is the group comprising glycerides, glycolipids, glycerophospholipids, isoprenoidlipids, sphingolipids, steroids, sterines or sterols, sulphur-containing lipids, lipids containing at least one carbohydrate residue, or other polar fatty derivatives, which are therefore all suitable candidates for said at least one first substance that forms said extended surfaces. More specifically, the selection is made amongst phosphatidylcholines, phosphatidyl-ethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins, sphingophospholipids, glycosphingolipids, cerebroside, ceramidpolyhexosides, sulphatides, sphingoplasmalogenes, or gangliosides.

25

Said extended surface-forming substance, which solves the problems outlined in the application, is preferably selected from the group of lipoids or lipids, with one or two, often different, fatty chains, especially with acyl-, alkanoyl-, alkyl-, alkylene-, alkenoyl-, alkoxy, or chains with omega-cyclohexyl-, cyclo-propane-, iso- or anteiso-branched segments, or any other

30

- 55 -

practically useful aliphatic chain. There is some preference to use lipids with *n*-decyl, *n*-dodecyl (lauryl), *n*-tetradecyl (myristyl), *n*-hexadecyl (cetyl), *n*-octadecyl (stearyl), *n*-eicosyl (arachinyl), *n*-docosyl (behenyl) or *n*-tetracosyl (lignoceryl), 9-*cis*-dodecenyl (lauroleyl), 9-*cis*-tetradecenyl (myristoleyl), 9-*cis*-
5 hexadecenyl (palmitoleinyl), 9-*cis*-octadecenyl (petroselinyl), 6-*trans*-octadecenyl (petroselaidinyl), 9-*cis*-octadecenyl (oleyl), 9-*trans*-octadecenyl (elaidinyl), 9-*cis*-eicosenyl (gadoleinyl), 9-*cis*-docosenyl (cetoleinyl) or 9-*cis*-tetracosoyl (nervonyl), *n*-decyloxy, *n*-dodecyloxy (lauryloxy), *n*-tetradecyloxy (myristyloxy), *n*-hexadecyloxy (cetyloxy), *n*-octadecyloxy (stearyloxy), *n*-
10 eicosyloxy (arachinyloxy), *n*-docosoyloxy (behenyloxy) or *n*-tetracosoyloxy (lignoceryloxy), 9-*cis*-dodecenyloxy (lauroleyloxy), 9-*cis*-tetradecenyloxy (myristoleyloxy), 9-*cis*-hexadecenyloxy (palmitoleinyloxy), 6-*cis*-octadecenyloxy (petroselinyloxy), 6-*trans*-octadecenyloxy (petroselaidinyloxy), 9-*cis*-octadecenyloxy (oleyloxy), 9-*trans*-octadecenyloxy
15 (elaidinyloxy), and 9-*cis*-eicosenyl (gadoleinyloxy), 9-*cis*-docosenyl (cetoleinyloxy) or 9-*cis*-tetracosoyl (nervonyloxy), *n*-decanoyloxy, *n*-dodecanoyloxy (lauroyloxy), *n*-tetradecanoyloxy (myristoyloxy), *n*-hexadecanoyloxy (palmitoyloxy) *n*-octadecanoyloxy (stearoyloxy), *n*-eicosanoyloxy (arachinoyloxy), *n*-docosanyloxy (behenoyloxy) and *n*-
20 tetracosanoyloxy (lignoceroyloxy), 9-*cis*-dodecenyloxy (lauroleyoyloxy), 9-*cis*-tetradecenoyloxy (myristoleoyloxy), 9-*cis*-hexadecenoyloxy (palmitoleinoyloxy), 6-*cis*-octadecenoyloxy (petroselinoyloxy), 6-*trans*-octadecenoyloxy (petroselaidinoyloxy), 9-*cis*-octadecenoyloxy (oleoyloxy), 9-*trans*-octadecenoyloxy (elaidinoyloxy), and 9-*cis*-eicosenoyloxy
25 (gadoleinoyloxy), 9-*cis*-docosenoyloxy (cetoleinoyloxy) and 9-*cis*-tetracosenoyloxy (nervonoyloxy) or the corresponding sphingosine derivative chains.

A preferred suitable solution to the problems outlined herein are amphipat
30 combinations in which said at least one second substance is a surface active

- 56 -

substance such as a surfactant / detergent. The latter is preferably selected from the group comprising nonionic, zwitterionic, anionic and cationic surfactants. It is preferred to use a surfactant with the solubility in a liquid suspending medium such as a polar liquid, in which the extended surfaces
5 are prepared, in the range 5×10^{-7} M to 10^{-2} M.

A long list of surfactants that qualify for the use in said quaternary suspensions are given herein before in the definition section.

10 For the solution of problems addressed by the application, charge-charge or charge-polar headgroup interactions amongst the involved amphipats may be important. If so, the following consideration can be made: if the at least one second substance is charged the at least one third substance can be is uncharged and if the at least one second substance is uncharged the at least
15 one third substance ideally should be charged; similar preference of combinations is also possible for the said at least one first and one second or for the said at least one first and one third substance, respectively. When at least one charged amphipat is used to prepare aggregates with at least three different components, the extended aggregate surface, formed by the at least
20 one first, one second and one third substance, at least one of which is charged, is preferably chosen to contain between 1% and 75% of the charged component. An even more favourable choice is to use combinations of at least one first, one second and one third substance, at least one of which is charged, that contain between 5% and 50% of the charged
25 component and most preferably between 10% and 30% of the charged component.

In some cases it is preferred to use combinations according to claims of this application such that contain a phosphatidylcholine, a
30 phosphatidylethanolamine-N-mono- or N-di-methyl, phosphatidic acid or its

- 57 -

- methyl ester, phosphatidylserine and/or phosphatidylglycerol as the surface-supporting at least one first substance and a lysophospholipid, especially a lysophosphatidic acid, lysomethylphosphatidic acid, lysophosphatidylglycerol, lysophosphatidylcholine, a partially N-methylated
- 5 lysophosphatidylethanolamine, or else a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, or a sufficiently polar sterol-derivative, or a suitable salt form of laurate, myristate, palmitate, oleate, palmitoleate, elaidate or some other pharmaceutically acceptable long-chain
- 10 chains, or a Triton, a long-chain fatty sulphonate, -sulphobetaine, -N-glucamide or -sorbitane (Arlacel or Span) surfactant, any of which can take the role of the at least one second or of at least one third substance, as the case may be, such second/third substance on its own forming less extended surfaces than the at least one first substance on its own.
- 15 Preferred combinations that conveniently solve the outlined problems may alternatively contain a biologically active amphipat, which can destabilise lipid membranes, as the least one second or one third substance, as the case may be, unless a surfactant different from the at least one second substance
- 20 or one third substance, but otherwise selected from similar surfactant classes, is selected for the purpose.

As a useful rule of the thumb, which can be applied to select a suitable at least one third or second substance, is preferably to select the solubility of

25 such substance in the liquid suspending medium, such as a polar liquid, to be between 10^{-6} M and 1 M.

For some embodiments it is preferred to seek such molecule taking the role of at least one third or second amphipat that adsorbs to the surface of lipid

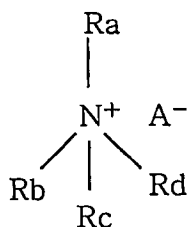
- 58 -

bilayers but is also well miscible with or reasonably soluble in the polar liquid in which said extended lipid bilayer surfaces are formed.

It is furthermore preferred, and practically useful, to use such drug or drug
 5 form that can take the role of as the at least one third or second substance, as the case may be, especially when this role is not taken by the at least one first and/or the at least one second or third substance, respectively. If so, such ionisation or salt form of the drug is chosen that serve the purpose best. To the effect, the bulk pH, electrolyte composition and concentration value,
 10 and in case of need also co-solvents including different short chain alcohols or other short chain polar amphipats are selected appropriately.

Drugs suitable for solving the problems sketched in this work can belong to the class of substituted ammonium compounds of the formula

15



(1)

20 in which a) Ra represents a hydrophobic group, and Rb, Rc, and Rd, independently of one another, each represents hydrogen, C1-C4-alkyl, 2-hydroxyethyl, allyl or cyclo-C3-C6-alkyl-C1-C3-alkyl, or two of the radicals Rb, Rc and Rd together represent C4- or C5- alkylene optionally interrupted by -HN-, -N(C1-C4-alkyl)-, -N(2-hydroxyethyl)- or by oxygen, or; b) Ra and
 25 Rb are two hydrophobic groups or together represent a hydrophobic group, and Rc and Rd, independently of one another, each represents hydrogen, C1-C4-alkyl, allyl or cyclo-C3-C6-alkyl-C1-C3-alkyl, or c) Ra, Rb and Rc

- 59 -

together represent a hydrophobic group, and Rd represents hydrogen or C1-C4-alkyl, and A⁻ represents the anion of a pharmaceutically acceptable acid, as a carboxylic acid salt of the formula

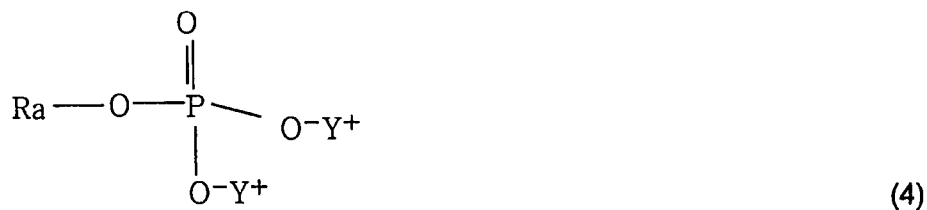


Ra representing a hydrophobic group and Y⁺ representing the cation of a pharmaceutically acceptable base, as an alpha-amino acid compound of the formula

10



15 In the above formula 3, Ra represents a hydrophobic group and Rb and Rc, independently of one another, each represents hydrogen or C1-C4-alkyl, as a phosphoric acid monoester of the formula



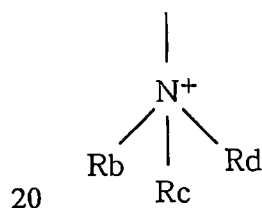
20

wherein Ra represents a hydrophobic group and Y⁺ represents the cation of a pharmaceutically acceptable base, or as an acid addition salt of a

- 60 -

compound having a hydrophobic group Ra and an imidazoline, imidasolidine or hydrasino group as hydrophilic group.

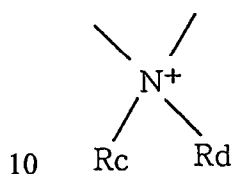
- In a substituted ammonium compound of the formula 1 that can be used as a medicament, in case a) the hydrophobic group Ra is an aliphatic hydrocarbon radical that can be interrupted by an oxygen or sulphur atom, may contain the groups -CO(=O)-, -O-C(=O)-, -C(=O)-NH-, -O-C(=O)-NH- or hydroxy, and can be substituted by from 1 to 3 optionally substituted, mono-cyclic, aliphatic or aromatic hydrocarbon radicals, by an optionally substituted, bi- or tri-cyclic, aromatic or partially saturated hydrocarbon radical, by an optionally substituted, monocyclic, aromatic, partially saturated or saturated heterocycle or by an optionally substituted, bi- or tri-cyclic, aromatic, partially saturated or benzo-fused heterocycle.
- The hydrophobic group Ra can also be an optionally substituted, monocyclic, aliphatic or aromatic hydrocarbon radical or a bicyclic, aliphatic or benzo-fused hydrocarbon radical. The hydrophilic group is, for example, a group of the formula



- wherein Rb, Rc, and Rd, independently of one another, each represents hydrogen, C1-C4-alkyl, for example methyl, ethyl, isopropyl or *n*-propyl, or 2-hydroxyethyl, or in which two of the radicals Rb, Rc, and Rd together represent piperidino, piperaziny, 1-methylpiperaziny, 1-(2-hydroxyethyl)-piperaziny or morpholino, and the other radical represents hydrogen.

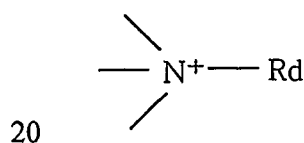
- 61 -

In a substituted ammonium compound of the formula 1 that can be used as a medicament, in case **b)** Ra and Rb are two hydrophobic groups, for example two aliphatic hydrocarbon radicals, which can be substituted by one or two optionally substituted, monocyclic, aliphatic or aromatic hydrocarbon radicals
 5 or by an optionally substituted, monocyclic, aromatic, partially saturated or saturated heterocycle, or Ra and Rb together represent an optionally substituted, monocyclic, aromatic, saturated, partially saturated or benzo-fused heterocycle. The hydrophilic group is a group of the formula



in which Rc and Rd, independently of one another each represents hydrogen or C1-C4-alkyl, preferably methyl.

15 In a substituted ammonium compound of the formula 1, which can be used as a medicament, in case **c)** Ra, Rb, and Rc form the hydrophobic group and together represent an optionally substituted, aromatic, partially saturated or benzo-fused heterocycle. The hydrophilic group is a group of the formula



in which Rd represents hydrogen or C1-C4-alkyl, preferably methyl.

25 A⁻ is the anion of a pharmaceutically acceptable acid, for example a mineral acid, for example the chloride, hydrogen sulphate or dihydrogen phosphate ion, the bromide or iodide ion, or the anion of an organic acid, for example a

- 62 -

lower alkanecarboxylic acid, for example the acetate ion, of an unsaturated carboxylic acid, for example the fumarate or maleate ion, of a hydroxy acid, for example the lactate, tartrate or citrate ion, or of an aromatic acid, for example the salicylate ion.

5

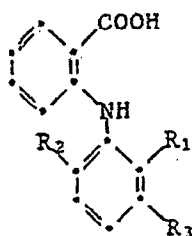
In a carboxylic acid salt of the formula 2, which can be used as a medicament, the hydrophobic group Ra is an aliphatic hydrocarbon radical that can be substituted by an optionally substituted, monocyclic, aromatic hydrocarbon radical or by an optionally substituted, bi- or tri-cyclic, aromatic
10 or partially saturated hydrocarbon radical, by an optionally substituted, monocyclic, aromatic or partially saturated heterocycle or by an optionally substituted, bi- or tri-cyclic, aromatic, partially saturated or benzo-fused heterocycle or by a steroid radical, or Ra is an optionally substituted, monocyclic, aromatic hydrocarbon radical, an optionally substituted, bi- or tri-
15 cyclic, aromatic or partially saturated hydrocarbon radical, an optionally substituted, monocyclic, aromatic or partially saturated heterocycle or an saturated or benzo-fused heterocycle.

The cation Y⁺ of a pharmaceutically acceptable base is, for example, an
20 alkali metal ion, for example a lithium, sodium or potassium ion, an alkaline earth metal ion, for example a magnesium or calcium ion, or an ammonium or mono-, di- or tri-C1-C4-alkylammonium ion, for example a trimethyl-, ethyl-, diethyl- or triethyl-ammonium ion, a 2-hydroxyethyl-tri-C1-C4-alkylammonium ion, for example choliny, or the cation of a basic amino acid,
25 for example lysine or arginine.

Carboxylic acid salts of the formula 2 having biological activity or carboxylic acids that can be converted into them by salt formation are, for example, salts of glucocorticoids that are esterified in the 21-position by a dicarboxylic
30 acid, for example methylprednisolone sodium succinate, prednisolone

- 63 -

- sodium succinate; short-term narcotics of the 3,20-dioxo-5 β -pregnane type that can be esterified by succinic acid, for example hydroxydione succinate sodium or 11,20-dioxo-3 α -hydroxy-5 α -pregnane, for example alphaxolone, or the 21-compound, for example alphadolone; salts of
- 5 choleritics, for example cholic acid salts or deoxycholic acid salts; analgesics, for example salts of substituted phenylacetic acids or 2-phenylpropionic acids, for example alclofenac, ibufenac, ibuprofen, clindanac, fenclorac, ketoprofen, fenoprofen, indoprofen, fenclofenac, diclofenac, flurbiprofen, pirprofen, naproxen, benoxaprofen, carprofen or cicloprofen; analgesically
- 10 active anthranilic acid derivatives, for example of the formula optionally substituted, bi- or tri-cyclic, aromatic,



(2.1)

15

- in which R1, R2 and R3 independently of one another, each represents hydrogen, methyl, chlorine or trifluoromethyl, for example mefenamic acid, flufenamic acid, tolfenamic acid or meclofenamic acid; analgesically active anilino-substituted nicotinic acid derivatives, for example miflumic acid,
- 20 clonixin or flunixin; analgesically active heteroarylacetic acids or 2-heteroarylpropionic acids having a 2-indol-3-yl or pyrrol-2-yl radical, for example indomethacin, oxmetacin, intrazol, acemetazin, cinmetacin, zomepirac, tolmetin, colpirac or tiaprofenic acid; analgesically active indenylacetic acids, for example sulindac; analgesically active
- 25 heteroaryloxyacetic acids, for example benzdac, prostanoic acids that

- 64 -

stimulate the smooth musculature, for example PGE2 (dinoprostone), PGF2alpha (dinoprost), 15 (S)-15-methyl-PGE2, 15 (S)-15-methyl-PGF2alpha, (carboprost), (\pm)15 (Xi)-15-methyl-13,14-dihydro-11-deoxy-PGE1 (deprostil), 15(S)-15-methyl-11-deoxy-PGE1 (doxaprost), 16,16-dimethyl-PGE2, 17-phenyl-18,19,20-trinor-PGF2alpha, 16-phenoxy-17,18,19,20-tetranor-PGF2alpha, for example cloprostenol or fluprostenol, or N-methylsulphonyl-15-phenoxy-17,18,19,20-tetranor-PGF2alpha (sulproston); bacteriostatics, for example salts of nalixidic acid derivatives, for example salts of nalixidic acid, cinoxacin, oxolinic acid, pironidic acid or

10 piperidic acid, penicillanic acid and cephalosporanic acid derivatives having antibiotic activity with 6 β - or 7 β -acylamino groups, which are present in fermentatively, semi-synthetically or totally synthetically obtainable 6 β -acylamino-penicillanic acid or 7 β -acylaminocephalosporanic acid derivatives or 7 β -acylaminocephalosporanic acid derivatives modified in the 3-position,

15 for example penicillanic acid derivatives that have become known under the names penicillin G or V, phenethicillin, propicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, flucloxacillin, cyclacillin, epicillin, mecillinam, methicillin, azlocillin, sulbenicillin, ticarcillin, mezlocillin, piperacillin, carindacillin, azidocillin or ciclazillin, or cephalosporin derivatives that have

20 become known under the names cefaclor, cefuroxime, cefazlur, cephradine, cefazolin, cephalixin, cefadroxil, cephaloglycin, cefoxitin, cephaloridine, cephsulodin, cefotiam, ceftazidime, cefonicid, cefotaxime, cefmenoxime, ceftizoxime, cephalothin, cephradine, cefamandol, cephanone, cephapirin, cefroxadin, cefatrizine, cefazedone, ceftriaxone or ceforanid, and other β -

25 lactam antibiotics, for example moxalactam, clavulanic acid, nocardicine A, sulbactam, aztreonam or thienamycin; or antineoplastics having a 4-[bis-(2-chloroethyl)-amino-phenyl]-butyric acid structure, for example chlorambucil, or antineoplastics having two carboxy groups, for example methotrexate.

- 65 -

Compounds of the formula 3 having a biological activity are, for example, neurotransmitters in which the hydrophobic group is methyl substituted by hydroxyphenyl, for example L-tyrosine, L-dopa, alpha-methyldopa or metirosine; thyroid hormones having iodine-substituted phenyl radicals, for example levo-tyrosine, diiodotyrosine or liothyronine; or anti-neoplastics having an amino acid structure, for example melphalen.

In a compound of the formula 4 having biological activity the non-polar, hydrophobic group Ra is a glucocorticoid radical and A⁺ is sodium, for example betamethasone disodium phosphate, dexamethasone disodium phosphate, cortisone phosphate, hydrocortisone phosphate, prednisolone disodium phosphate or paramethasone-21-disodium phosphate, Salt-type compounds having a hydrophobic group and an imidazoline, imidazolidine or hydrazino group as hydrophilic group are, for example, salts of anti-depressantly active hydrazine derivatives, for example iproniazid, nialamide, isocarboxazid, phenelzine, pheniprazine, mebanazine or fenoxypipazine; a-sympathomimetics having an imidazoline structure, for example naphazoline, tetraizolin, tramazoline, xylo-metazoline or oxymetazoline; n-sympatholytics having an imidazoline structure, for example phentolamine or tolazoline, or centrally active antihypertensives having an imidazoline structure, for example clonidine, tolondine or flutondine; or vasodilators having a hydrazino group, for example dihydralazine, hydralazine or picodralazine.

The said at least one third amphipatic substance in said combination, which acts as a drug, can be an adrenocorticostatic, a β -adrenolytic, an androgen, an antiandrogen, an antiparasitic, an anabolic, an anaesthetic, an analgesic, an analeptic, an antiallergic, an antiarrhythmic, an antiarterosclerotic, an antiasthmatic, a bronchospasmolytic, an antibiotic, an antidrepressive, an antipsychotic, an antidiabetic, an antidote, an antiemetic, an antiepileptic, an antifibrinolytic, an anticonvulsive, an anticholinergic, an enzyme, a coenzyme

- 66 -

- or corresponding inhibitor, an antihistaminic, an antihypertonic, a biological inhibitor of drug activity, an antihypotonic, an anticoagulant, an antimycotic, an antimyasthenic, an agent against Morbus Parkinson or Morbus Alzheimer, an antiphlogistic, an antipyretic, an antirheumatic, an antiseptic, a respiratory
- 5 analeptic or a respiratory stimulant, a broncholytic, a cardiotonic, a chemotherapeutic, a coronary dilatator, a cytostatic, a diuretic, a ganglium-blocker, a glucocorticoid, an antiflow agent, a haemostatic, a hypnotic, an immunoglobuline or its fragment, an immunologically active substance, a bioactive carbohydrate, a bioactive carbohydrate derivative, a contraceptive,
- 10 an anti-migraine agent, a mineralo-corticoid, a morphine-antagonist, a muscle relaxant, a narcotic, a neurotherapeutic, a neuroleptic, a neurotransmitter or its antagonist, a small peptide, a small peptide derivative, an ophthalmic, a sympaticomimetic or a sympathicolytic, a para-sympaticomimetic or a para-sympathicolytic, a psoriasis drug, a
- 15 neurodermitis drug, a mydriatic, a psychostimulant, a rhinologic, a sleep-inducing agent or its antagonist, a sedating agent, a spasmolytic, tuberculostatic, an urologic agent, a vasoconstrictor or vasodilatator, a virustatic, a wound-healing substance, or a combination of aforesaid agents.
- 20 When a drug is used as said at least one second or third component, its content is preferably chosen to be between 0.1 rel.% and 60 rel.% compared to the total mass of all three said substances forming said extended surfaces. Somewhat narrower, and more preferred, choice is to use between 0.5 rel.% and 50 rel.% and most favourably between 1 rel.% and 40 rel.% compared to
- 25 the total mass of all three said substances that form said extended surfaces.

Said at least one third substance in amphipat combination, which solves the outlined problems, can be a low molecular weight immunomodulator, a biocatalyst, a co-enzyme, a hormone, or a low molecular weight agonist or

30 antagonist of some biologically important substance action.

- 67 -

Any low to intermediate weight polypeptide with membrane destabilising properties is also useful in the context of this invention, if included into said combinations in suitable form and concentration.

5

In one important aspect of the present invention as described above, one of the at least one second or at least on third amphipatic component is a non-steroidal anti-inflammatory drug (NSAID).

10

In one important aspect of the present invention, the invention provides preparations, based on a suspension of extended surface aggregates in a liquid medium comprising at least one first amphipatic component; at least one second amphipatic component; at least one third amphipatic component, the first amphipatic component being a vesicle membrane forming lipid

15

component, the second and third component being membrane destabilising components, wherein the third component is a non-steroidal anti-inflammatory drug (NSAID) such that said aggregates are capable of penetrating semi-permeable barriers with pores at least 50% smaller than the average aggregate diameter before the penetration without changing their

20

diameter by more than 25%.

It is another aspect of the invention suspensions of extended surface aggregates in a liquid medium are provided, comprising: at least one first amphipatic component; at least one second amphipatic component; at least one third amphipatic component; the first amphipatic component being an aggregate, typically a membrane, forming lipid component; the second and third component being aggregate, typically membrane, destabilising components; wherein the third component is a NSAID, such that the extended surfaces formed by the first and second component alone or else

30

by the first and third component alone, the second or third component,

- 68 -

respectively, being present at a relative concentration X, have a lower propensity to overcome barriers with pores having a diameter at least 50% smaller than the average aggregate diameter, before the pore crossing, than the extended surfaces formed by the first, second and third component

5 together, if the second and third components are present at or below the combined relative concentration of X. More specifically, this e.g. means that:

a) said extended surfaces formed by the first and second component alone, the second component being present at a relative concentration X, have a lower propensity to overcome barriers with the pores at least 50% smaller

10 than the average aggregate diameter before the pore crossing than the extended surfaces formed by the first, second and third component, if the second and third components are present at or below a combined concentration of X compared to the concentration of the first component; or

else b) such extended surfaces formed by the first and third component

15 alone, the third component being present at a relative concentration X, have a lower propensity to overcome barriers with the pores at least 50% smaller than the average aggregate diameter before the pore crossing than

extended surfaces formed by the first, second and third component, the second and third components together being present at or below a

20 concentration of X compared to the concentration of the first component.

In yet another aspect of the invention extended surface aggregates suspended in a liquid medium are provided, comprising: at least one first amphipatic component; at least one second amphipatic component; at least

25 one third amphipatic component; the first amphipatic component being a membrane forming lipid component; the second and third component being membrane destabilising components, such that the third component is a NSAID; and the inclusion of the second or third component to an otherwise two amphipatic-component mixture increases the suspension flux (at a given

30 transbarrier pressure, Δp) through the pores at least 50% smaller than

the average aggregate diameter before the penetration in comparison with the flux of the suspension containing aggregates comprising merely the first and second or the first and third components, respectively. More specifically, the inclusion of the third component increases the flux of said suspension
5 compared with the flux of the suspension containing simpler aggregates comprising merely the first and second component or else the inclusion of the second component increases the flux of said suspension compared with the flux of the suspension containing simpler aggregates comprising merely the first and third component.

10

In a further aspect of this invention extended surface aggregates suspended in a liquid medium comprise: at least one first amphipatic component; at least one second amphipatic component; at least one third amphipatic component; the first amphipatic component being a membrane forming lipid component;
15 the second and third component being membrane destabilising components, such that the third component is a NSAID and that the addition of the second or third component to an originally two component mixture increases aggregate adaptability of the resulting extended surface aggregates with at least three components compared to the aggregates containing respective
20 combinations of the first and the third or the first and the second components alone. More specifically, the inclusion of the third component increases the aggregate adaptability of an extended surface aggregate comprising the first and second components alone; or else, the inclusion of the second component increases the aggregate adaptability of an extended surface
25 aggregate comprising the first and third components alone.

Yet another aspect of this invention provides extended surface aggregates suspended in a liquid medium, comprising: at least one first amphipatic component; at least one second amphipatic component; at least one third
30 amphipatic component; the first amphipatic component being a membrane

- 70 -

forming lipid component; the second and third component being aggregate destabilising components, such that the third component is an NSAID; and the inclusion of the second or third component to an otherwise two amphipatic component mixture lowers the driving pressure required for aggregate penetration of pores at least 50% smaller than the average aggregate diameter before the penetration in comparison with the aggregates comprising merely the first and second or the first and third components, respectively. More specifically, the inclusion of the second component lowers the driving pressure required for aggregate penetration of pores at least 50% smaller than the average aggregate diameter before the penetration in comparison with the aggregates comprising merely the first and third components; alternatively, the inclusion of the third component lowers the driving pressure required for aggregate penetration of pores at least 50% smaller than the average aggregate diameter before the penetration in comparison with the aggregates comprising merely the first and second components.

It is a further aspect of this invention to provide extended surface aggregates suspended in a liquid medium, comprising: at least one first amphipatic component; at least one second amphipatic component; at least one third amphipatic component; the first amphipatic component being a membrane forming lipid component; the second and third component being membrane destabilising components, such that the third component is an NSAID and the inclusion of the second or third component to an otherwise two amphipatic component mixture increases the deformability of extended surface aggregates compared with the aggregates comprising merely the first and second or the first and third component, respectively. More specifically, the inclusion of the third component increases the deformability of the extended surface aggregates compared with the aggregates comprising merely the first and second component; alternatively, the inclusion of the second component

- 71 -

increases the deformability of the extended surface aggregate compared with the aggregates comprising merely the first and third component.

The invention teaches preparation and use of said extended surface
5 aggregates in the form of membrane-enclosed, liquid-filled vesicles, whereby said first component is a membrane-forming lipid, and said second and third components are membrane-destabilising components.

The invention includes suspensions of extended surface aggregates in a
10 liquid medium comprising: at least one first amphipatic component; at least one second amphipatic component; at least one third amphipatic component; the first amphipatic component being a membrane forming lipid component; the second and third component being membrane destabilising components, such that the third component is a non-steroidal anti-inflammatory drug
15 (NSAID) and such that said extended surface aggregates can penetrate intact mammalian skin, thus increasing NSAID concentration in the skin and/or increasing the reach of NSAID distribution below the skin, in comparison with the result of the same NSAID application in a solution on the skin. In a special version of said suspensions, said extended surface
20 aggregates are membrane-enclosed, liquid-filled vesicles, said first component is a membrane-forming lipid, and said second and third components are membrane-destabilising components.

It is also an aspect of this invention to provide said suspensions wherein the
25 third component is an NSAID, as defined above, most preferably is ketoprofen, ibuprofen, diclofenac, indomethacin, naproxen or piroxicam. To prepare said suspensions with these or other NSAID ingredients, the first, stable membranes forming, component is selected from the group consisting of lipids, lipoids, from a biological source, corresponding synthetic lipids or
30 lipoids, or modifications thereof. In this context it is preferable to choose

- 72 -

- amongst glycerides, glycolipids, glycerophospholipids, isoprenoidlipids, sphingolipids, steroids, sterines or sterols, sulphur-containing lipids, lipids containing at least one carbohydrate residue, or other polar fatty derivatives. Specifically, the preferred choice are the groups of phosphatidylcholines,
- 5 phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins, sphingophospholipids, glycosphingolipids, cerebroside, ceramidpolyhexosides, sulphatides, sphingoplasmalogenes, or gangliosides.
- 10 To manufacture a pharmaceutical formulation, it may be advisable or necessary to prepare the product in several steps, changing temperature, pH, ion strength, individual component (e.g. membrane destabiliser, formulation stabiliser or microbicide) or total lipid concentration, or suspension viscosity during the process.
- 15 Quite detailed recommendations on the preparation of said combinations is given in EP 0 475 160 and USP 6 165 500, which are herewith included by reference, using filtering material with pore diameters between 0.01 μm and 0.1 μm , more preferably with pore diameters between 0.02 μm and 0.3 μm
- 20 and even more advisable filters with pore diameters between 0.05 μm and 0.15 μm to homogenise final vesicle suspension, when filtration is used for the purpose. Other methods of mechanical homogenisation or for lipid vesicle preparation known in the art are useful as well.
- 25 A list of relevant and practically useful thickening agents is given e.g. in PCT/EP98/08421, which also suggests numerous interesting microbicides and antioxidants; the corresponding sections of PCT/EP98/08421 are therefore included into the present application by reference. Practical experiments have confirmed that sulphites, such as sodium sulphite,
- 30 potassium sulphite, bisulphite and metasilphite; and potentially other water

- soluble antioxidants, which also contain a sulphur or else a phosphorus atom (e.g. in pyrosulphate, pyrophosphate, polyphosphate), erythorbate, tartrate, glutamate, etc. or even L-tryptophan), ideally with a spectrum of activity similar to that of sulphites) offer some anti-oxidative protection to said
- 5 formulations, final selection being subject to regulatory constraints. Any hydrophilic antioxidant should always be combined with a lipophilic antioxidant, however, such as BHT (butylated hydroxytoluene) or BHA (butylated hydroxyanisole).
- 10 It is a further aspect of this invention to teach that the first suspension component is preferably selected amongst lipids, , with one or two, not necessarily identical, fatty chains, especially with acyl-, alkanoyl, alkyl-, alkylene-, alkenoyl-, alkoxy, or chains with omega-cyclohexyl-, cyclopropane-, iso- or anteiso-branched segments, or the corresponding chains
- 15 mixtures. Useful chains include *n*-decyl, *n*-dodecyl (lauryl), *n*-tetradecyl (myristyl), *n*-hexadecyl (palmityl), *n*-octadecyl (stearyl), *n*-eicosyl (arachinyl), *n*-docosyl (behenyl) or *n*-tetracosyl (lignoceryl), 9-*cis*-dodecenyl (lauroleyl), 9-*cis*-tetradecenyl (myristoleyl), 9-*cis*-hexadecenyl (palmitoleinyl), 9-*cis*-octadecenyl (petroselinyl), 6-*trans*-octadecenyl (petroselaidinyl), 9-*cis*-octadecenyl (oleyl), 9-*trans*-octadecenyl (elaidinyl), 9-*cis*-eicosenyl (gadoleinyl), 9-*cis*-docosenyl (cetoleinyl) or 9-*cis*-tetracosyl (nervonyl), *n*-decyloxy, *n*-dodecyloxy (lauryloxy), *n*-tetradecyloxy (myristyloxy), *n*-hexadecyloxy (cetyloxy), *n*-octadecyloxy (stearyloxy), *n*-eicosyloxy (arachinyloxy), *n*-docosyloxy (behenyloxy) or *n*-tetracosyloxy
- 20 (lignoceryloxy), 9-*cis*-dodecenyloxy (laurolexyloxy), 9-*cis*-tetradecenyloxy (myristoleyloxy), 9-*cis*-hexadecenyloxy (palmitoleyloxy), 6-*cis*-octadecenyloxy (petroselinyloxy), 6-*trans*-octadecenyloxy (petroselaidinyloxy), 9-*cis*-octadecenyloxy (olexyloxy), 9-*trans*-octadecenyloxy (elaidinyloxy), and 9-*cis*-eicosenyl (gadoleyloxy), 9-*cis*-docosenyl (cetoleyloxy) or 9-*cis*-tetracosyl (nervonyloxy), *n*-decanoyloxy, *n*-
- 25 (cetoleyloxy) or 9-*cis*-tetracosyl (nervonyloxy), *n*-decanoyloxy, *n*-
- 30 (cetoleyloxy) or 9-*cis*-tetracosyl (nervonyloxy), *n*-decanoyloxy, *n*-

- 74 -

- dodecanoyloxy (lauroyloxy), *n*-tetradecanoyloxy (myristoyloxy), *n*-hexadecanoyloxy (palmitoyloxy) *n*-octadecanoyloxy (stearoyloxy), *n*-eicosanoyloxy (arachinoyloxy), *n*-*n*-docosoanyloxy (behenoyloxy) and *n*-tetracosanoyloxy (lignoceroyloxy), 9-*cis*-dodecenyloxy (lauroleoyloxy), 9-*cis*-
5 tetradecenoyloxy (myristoleoyloxy), 9-*cis*-hexadecenoyloxy (palmitoleinoyloxy), 6-*cis*-octadecenoyloxy (petroselinoyloxy), 6-*trans*-octadecenoyloxy (petroselaidinoyloxy), 9-*cis*-octadecenoyloxy (oleoyloxy) , 9-*trans*-octadecenoyloxyelaidinoyloxy), and 9-*cis*-eicosenoyloxy (gadoleinoyloxy), 9-*cis*-docosenoyloxy (cetoleinoyloxy) and 9-*cis*-
10 tetracosenoyloxy (nervonoyloxy) or the corresponding sphingosine derivative chains, or corresponding two double bonds combinations, especially in the sequence 6,9-*cis*, 9,12-*cis* or, in case, 12,15-*cis* or else the related three double bonds combinations, especially in the sequence, 6,9,12-*cis*, or 9,12,15-*cis* are preferable. A preferred choice in case of
15 phosphatidylcholines of biological, and preferably plant, origin, is to use the lipids extracted from soy (bean), coconut, olive, safflower or sunflower, linseed, evening primrose, primrose, or castor oil, and the like, other biological sources of general availability, such as eggs, also being an option.
- 20 According to the invention the second suspension component, which tends to destabilise lipid membranes, is preferably a surfactant. The selected surfactant can belong to the group of nonionic, zwitterionic, anionic and cationic surfactants. Preferentially, any such surfactant is chose to have solubility in the liquid medium ranging from about 5×10^{-7} M to about 10^{-2} M.
- 25 An alternative definition of surfactants useful for the use in said suspensions of extended surface aggregates relates to hydrophilicity-lipophilicity ratio (HLB), which should be between 10 and 20, preferably between 12 and 18 and most preferred between 13 and 17. A good choice of non-ionic surfactants according to this invention are polyethyleneglycol-sorbitan-long
30 fatty chain esters, from polyethyleneglycol-long fatty chain esters or -ethers

- 75 -

and from polyhydroxyethylen-long fatty chain esters or -ethers; preferably, the number of ethyleneglycol or hydroxyethylen units per such surfactant molecule is selected to be in the range 6 to 30, more conveniently to be between 8 and 25 and most and typically to be between 12 to 20.

- 5 Alternatively, non-ionic phospholipids with water solubility similar to that of said non-ionic surfactants, can be used to the same effect. Examples include lyso-phospholipids, certain phosphatidylglycerols, phospholipids with one long and one short (C1-C6) chain, etc. In order to ensure sufficient fluidity of resulting complex extended surface aggregates, the hydrophobic chain
- 10 attached to such polar groups is preferentially chosen to be sufficiently short or to be unsaturated; polyethyleneglycol-sorbitan-monolaurate and polyethyleneglycol-sorbitan-monooleate, polyethyleneglycol-monolaurate and polyethyleneglycol-monooleate or polyethyleneglycol-monolaurate-ether and polyethyleneglycol-monooleate-ether are good choices in this respect. More
- 15 specifically, it is preferable in the context of this invention, to use a surfactant which is polyethyleneglycol-sorbitan-monooleate or monolaurate (e.g. Tween 80 or Tween 20) or else is polyethyleneglycol-oleate or laurate (i.e. POE-oleate or POE-laurate) or else is polyethyleneglycol-oleyl-ether or lauryl-ether, with 6 to 30, more preferably 8 to 15 and most preferred 12 to 20
- 20 ethyleneglycol (i.e. oxyethylene or OE) units per surfactant molecule.

- It is another aspect of this invention to combine, in said suspension, a phosphatidylcholine, as the first component, and ketoprofen, diclofenac, ibuprofen, indomethacin, naproxen, or piroxicam, as the third, NSAID,
- 25 component. A preferred choice is the combination of soy phosphatidylcholine, as the first, and of ketoprofen, diclofenac, ibuprofen, indomethacin, naproxen or piroxicam as the third component.

- In a preferred embodiment of the invention, the second component is a non-
- 30 ionic surfactant, such as a polyethyleneglycol-sorbitan-long fatty chain ester,

- 76 -

- a polyethyleneglycol-long fatty chain ester or a polyethyleneglycol-long fatty chain ether or else the corresponding surfactant with a polyhydroxyethylene polar group. A preferred choice is the use of polyethyleneglycol-sorbitan-monooleate or -laurate, of polyethyleneglycol-monooleate or -laurate, or else
5 of polyethyleneglycol-oleyl-ether or -lauryl-ether as the second component. In the resulting suspension, the second component is preferably chosen to carry a polyethyleneglycol (PEG or POE) polar head with 6 to 30, more preferably 8 to 15 and most preferred 12 to 20 ethyleneglycol (i.e. oxyethylene or OE) units per surfactant molecule. Alternatively, non-ionic
10 phospholipids, with water solubility similar to that of said non-ionic surfactants, can be used for similar purpose. Moreover, the hydrophobic chains are always chosen to be in a fluid state or at least to be compatible with such state of a carrier aggregate.
- 15 In another preferred embodiment of this invention is to provide said suspensions such that contain aggregates with an average diameter before the aggregates penetrate the pores at least 40% larger than the average pore diameter in the barrier of interest.
- 20 In a preferred embodiment of the invention, extended surface aggregates are proposed to have an average aggregate diameter that is at least 50% larger before pore penetration than the average pore diameter. Preferably, the average aggregate diameter before the aggregates penetrate the pores is at least 70%, even more preferably is at least 100% and most preferably is at
25 least 150% larger than the average pore diameter.

Another aspect of the invention is to provide suspensions in which the first component and the second component differ in solubility in the liquid medium at least 10-fold, on the average. The preferred difference in solubility
30 between the second and third component is, on the average, at least 2-fold.

- 77 -

In a further preferred embodiment of the invention said suspension comprises a total dry mass of the at least three amphipatic components between 0.01 weight-% and 50 weight-%. A more preferred choice is to keep this total
5 dry mass between 0.1 weight-% and 40 weight-%, better to keep even it between 0.5 weight-% and 30 weight-% and best to select the total dry mass of the three amphipatic components between 1 weight-% and 15 weight-%, at the time of formulation preparation and/or application.

10 Yet another aspect of the invention is to provide suspensions of extended surface aggregates, formed by the three components, with an average curvature corresponding to the average aggregate diameter between 15 nm and 5000 nm, preferably between 30 nm and 1000 nm, more preferred between 40 nm and 300 nm and most preferred between 50 nm and 150 nm.

15 A different aspect of the invention is to advocate using suspensions of extended surface aggregates that contain a lower aliphatic alcohol with a membrane partition coefficient and polarity such that the alcohol, as the at least one further second component, takes the role of a membrane
20 destabilising component. Alcohols that potential qualify for such use include mono-alcohols, diols, or to some extent polyols, of low carbon number (C1-C6), and ethers thereof; preferred examples are ethanol, isopropanol, 1,2-propanediol, propylene glycol, glycerol, ethylene glycol, ethylene glycol monoethyl or monobutyl ether, propylene glycol monomethyl, monoethyl or
25 monobutyl ether, diethylene glycol monomethyl or monoethyl ether and analogous products. The preferred choice are simple alcohols, short chain diols or a short chain triols, preferably with the OH-residues grouped together, corresponding methyl-, ethyl-, or butyl-derivatives also being a possibility. This includes especially n-propanol, iso-propanol, or 2-propanol,
30 n-butanol, or 2-butanol, 1,2-propanediol, 1,2-butanediol; if ethanol is used,

the total alcohol and lipid concentration are selected such that practically useful ethanol association with a pore penetrating aggregate is ensured. Specifically, if used individually to increase extended surface aggregate adaptability, ethanol, n-propanol, 2-propanol, butanol, and benzyl alcohol are
5 preferably used at concentrations up to 15 w-%, 10 w-%, 8 w-%, 4 w-% and 2 w-%, respectively, in case of an initially 10 w-% total lipid suspension. The published water-membrane partition coefficients for other alcohols can be used together with these recommendations to select preferred concentration of other alcohols, or of alcohol combinations.

10

An important further aspect of the invention is to propose pharmaceutical preparations comprising suspensions according to the invention. A very convenient and preferred form of aggregates in such suspension is that of liquid-filled vesicles in an aqueous medium, the vesicles being enclosed by
15 membranes formed from at least one lipid component and comprising at least two membrane destabilising components one of which is an NSAID, whereby the extended surfaces formed by the first and second component alone or else by the first and third component alone, the second or third component, respectively, being present at a relative concentration X, have a lower
20 propensity to overcome barriers with pores at least 50% smaller than the average aggregate diameter before the pore crossing than the extended surfaces formed by the first, second and third component together, if the second and third components are present at or below the combined relative concentration of X.

25

It is also an important aspect of the invention, to teach pharmaceutical preparations comprising a suspension of liquid-filled vesicles in an aqueous medium, the vesicles being enclosed by membranes formed from at least one lipid component and comprising at least three membrane destabilising
30 components, whereby the membrane destabilising components comprise at

- 79 -

least one surfactant, at least one lower aliphatic alcohol and at least one non-steroidal anti-inflammatory drug; such that the membrane destabilising components increase the adaptability of the resulting extended surface aggregates with at least three components compared to the aggregates
5 containing respective combinations of the first and the third or the first and the second components alone.

It is a further aspect of the invention to provide pharmaceutical preparations comprising a suspension of liquid-filled vesicles in an aqueous medium, the
10 vesicles being enclosed by membranes formed from at least one lipid component and comprising at least three membrane destabilising components, whereby the membrane destabilising components comprise at least one surfactant, at least one lower aliphatic alcohol and at least one non-steroidal anti-inflammatory drug, such that the membrane destabilising
15 components increase the deformability of the vesicles and the vesicles are capable of penetrating barriers with pores at least 50% smaller than the average aggregate diameter before the penetration without changing their diameter by more than 25%.

20 It is a different aspect of the invention to provide pharmaceutical preparations comprising a suspension of liquid-filled vesicles in an aqueous medium, the vesicle being enclosed by membranes formed from at least one lipid component and comprising at least three membrane destabilising components, whereby the membrane destabilising components comprise a
25 surfactant, a lower aliphatic alcohol and a non-steroidal anti-inflammatory drug, whereby the membrane destabilising components increase the vesicle ability to penetrate mammalian skin and thus increase the reach of NSAID distribution in the skin, and beyond, in comparison with the result of an NSAID application in a solution on the skin.

30

A preferred embodiment of the invention provides vesicle containing pharmaceutical preparations in which a phosphatidylcholine takes the role of first component and an NSAID, such as ketoprofen, diclofenac, ibuprofen indomethacin, naproxen, or piroxicam is the third component.

5

In another preferred embodiment of the invention pharmaceutical preparations contain a nonionic surfactant, preferably a polyethyleneglycol-sorbitan-long fatty chain ester, a polyethyleneglycol-long fatty chain ester or a polyethyleneglycol-long fatty chain ether, the polyethyleneglycol chain being potentially replaced by a polyhydroxyethylene polar group. Specifically preferred are polyethyleneglycol-sorbitan-monooleate (e.g. Tween 80) or -laurate (e.g. Tween 20), or else polyoxyethylene-monooleate (e.g. Cithrol 10MO / Chemax E-1000) or -laurate (e.g. Cithrol 10ML) or else polyoxyethylene-oleyl-ether (e.g. Brij 98) or -lauryl-ether (e.g. Brij 35).

10

Alternatively, non-ionic phospholipids, with water solubility similar to that of said non-ionic surfactants, are used as a preferred nonionic surfactant.

15

In a related embodiment of the invention, said pharmaceutical preparations contain an alcohol, which preferably is selected from *n*-propanol, iso-propanol, 2-propanol, *n*-butanol or 2-butanol, 1,2-propanediol, or 1,2-butanediol, a methyl- or ethyl-derivative thereof, or ethanol. When ethanol is used, the total alcohol and lipid concentration is chosen to ensure a practically useful ethanol association with a pore penetrating aggregate.

20

It is also an aspect of the invention to provide such pharmaceutical preparations that are characterised by the bulk pH value above the logarithm of the apparent dissociation constant (pKa) of the NSAID in a solution and in the extended surface aggregates, the latter pKa being higher than the former. Preferably, the bulk pH value is selected to be between 0.2 pH and 2.2 pH units above pKa of the NSAID in an extended surface aggregate, more

25

30

- 81 -

preferably is between 0.5 pH and 1.9 pH units above this pKa and ideally is between 0.8 pH and 1.6 pH units above such pKa. Specifically, for the particularly interesting NSAIDs, ketoprofen or ibuprofen, the selected bulk pH is between 6.4 and 8.3, more preferably is between 6.7 and 8 and most preferably is between 7 and 7.7; for diclofenac the preferred bulk pH is between 6.2 and 8.1, more preferably is between 6.5 and 7.8 and most preferably is between 6.8 and 7.5; for naproxen the corresponding preferred pH value is between 6.3 and 8.2, more preferably is between 6.6 and 7.9 and most preferably is between 6.9 and 7.6; for piroxicam the choice of suspension bulk pH should be between 7.2 and 9, more preferably between 7.3 and 8.5 and most preferably between 7.4 and 8.2.

It is another aspect of the invention to select the bulk ionic strength of said pharmaceutical preparation to be between 0.005 and 0.3, even better between 0.01 and 0.2 and best between 0.05 and 0.15.

In preferred embodiment of the invention the said pharmaceutical formulation has viscosity between 50 mPa s and 30.000 mPa s. Preferably, the formulation viscosity is chosen to be between 100 mPa s and 10.000 mPa s, even better between 200 mPa s and 5000 mPa s, and most preferred between 400 mPa s and 2000 mPa s. To achieve such viscosity, at least one thickening agent may be added to said pharmaceutical formulation, precise choice and concentration of such agent depending on the ambient temperature, pH, ion strength, presence of other viscosity modifiers (such as glycerol), etc..

Thickening agents that are useful in the context of present invention are typically pharmaceutically acceptable hydrophilic polymers, including partially etherified cellulose derivatives, such as carboxymethyl-, hydroxyethyl-, hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely

- 82 -

synthetic hydrophilic polymers, including polyacrylates, polymethacrylates, poly(hydroxyethyl)-, poly(hydroxypropyl)-, poly(hydroxypropylmethyl)methacrylate, polyacrylonitriles, methallyl-sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols, 5 polyethylene glycol-lactides, polyethylene glycol-diacrylates, polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamide), poly(propylene fumarate-co-ethylene glycol), poloxamers, polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicone; natural gums, such as alginates, carrageenan, guar-gum, gelatine, tragacanth, (amidated) pectin, 10 xanthan, chitosan collagen, agarose; mixtures and further derivatives or copolymers thereof and/or other biologically acceptable polymers.

Most of such thickening agents in said pharmaceutical preparation are employed in weight concentration between 0.1 w-% and 10 w-%.

15

For the use of pharmaceutical formulations of the invention, the following hydrophilic polymer are preferred, amongst others: partially etherified cellulose derivatives, such as carboxymethyl -, hydroxyethyl-, hydroxypropyl-cellulose or amongst completely synthetic hydrophilic polymers from the 20 class of polyacrylates, such as polymethacrylates, poly(hydroxyethyl)-, poly(hydroxypropyl)-, poly(hydroxypropylmethyl)methacrylate, especially Carbopols.

Most preferably, such formulation thickeners are chosen from the group of 25 polysaccharides and derivatives thereof that are commonly used on the skin, including e.g. hyaluronic acid or hydroxypropylmethylcellulose; particularly preferably choices from the group of polyacrylates include the group of Carbopols, such as Carbopol grades 974, 980, 981, 1 382, 2 984, 5 984, in each case individually or in combination. In case of Carbopols (e.g. Carbopol 30 974), used to thicken the suspension-based multicomponent formulations for

improving NSAID delivery through permeability barriers and the skin, the polymer concentration preferably is selected to be between 0.3 w-% and 5 w-%, better between 0.5 w-% and 3w-% and best between 0.75 w-% and 1.75 w-%. Manufacturer's recommendations for obtaining certain viscosity can be
5 combined with these guiding concentrations to use other polymers or polymer combinations in a formulation for similar purpose.

It is another preferred embodiment of the invention to use of at least one antioxidant in said pharmaceutical formulations, which is typically selected
10 amongst synthetic phenolic compounds and their derivatives, the quinone-group containing substances, aromatic amines, ethylenediamine derivatives, various phenolic acids, tocopherols and their derivatives, including the corresponding amide and thiocarboxamide analogues; ascorbic acid and its salts; primaquine, quinacrine, chloroquine, hydroxychloroquine, azathioprine,
15 phenobarbital, acetaminophen); aminosalicyclic acids and derivatives; methotrexate, probucol, sulphur or phosphate atom containing anti-oxidants, thiourea; chellating agents, miscellaneous endogenous defence systems, and enzymatic antioxidants, etc.. Preferred are combinations of at least two antioxidants, one being lipophilic, such as butylated hydroxyanisol (BHA),
20 butylated hydroxytoluene (BHT), di-tert-butylphenol, or tertiary butylhydroquinone (TBHQ), and the other being hydrophilic, such as a chellating agent, especially EDTA, GDTA, or desferral, and/or is a sulphite, such as sodium or potassium metabisulphite, a pyrosulphate, pyrophosphate or polyphosphate. The butylated hydroxyanisol (BHA) or
25 hydroxytoluene (BHT) are typically used at concentrations between 0.001 w-% and 2 w-%, more preferably between 0.0025 w-% and 0.2 w-%, and most preferably is between 0.005 w-% and 0.02 w-%; EDTA or GDTA concentration is typically chosen between 0.001 w-% and 5 w-%, preferably between 0.005 w-% and 0.5 w-%, more preferably between 0.01 w-% and 0.2
30 w-% and most preferably between 0.05 and 0.975 w-%; a sulphite, such as

- 84 -

sodium or potassium metabisulphite is used preferably used in concentration range between 0.001 w-% and 5 w-%, more preferably between 0.005 w-% and 0.5 w-%, and most preferably between 0.01 w-% and 0.15 w-%.

- 5 In preferred embodiments of the invention pharmaceutical preparations contain at least one microbicide in concentration range between 0.1 w-% and 5 w-%, as is required for proper action and as is acceptable by a regulatory body.
- 10 Likewise, it is preferred according to of the invention that molar concentration ratio of the phospholipid component, which forms stable lipid membranes, and of the third, surfactant-like component, which destabilises such membranes, in said pharmaceutical preparations should be between 40/1 and 4/1. More preferably such a molar ratio is between 30/1 and 7.5/1, the
- 15 ratios between 20/1 and 10/1 being most preferred.

It is a further aspect of the invention to suggest composing a kit, comprising, in a tube or otherwise packaged form, at least one dose of the pharmaceutical preparation containing an NSAID associated with the

20 aggregates suitable for overcoming biological barriers such as the skin.

It is another aspect of the invention to propose a method for treating peripheral pain and/or inflammation by applying said pharmaceutical preparation on the skin of a warm blooded mammal.

25

A further aspect of the invention is to select different formulation doses per area to control the depth of NSAID delivery, if desirable using a non-occlusive patch for the purpose.

30 In a special embodiment of the invention at least one dose of an NSAID in

- 85 -

said pharmaceutical formulation is applied, and the application is repeated several, e.g. up to five times per day, if necessary, the preferred choice being two applications per day.

- 5 In presently preferred pharmaceutical preparations the first, i.e. phospholipid, component and the third, i. e. NSAID, components are present in the molar range between 10/1 and 1/1. A more preferred range molar range of these two components is between 5/1 and 2/1, or even between 4/1 and 2.5/1 and the most preferred composition have phospholipid/NSAID molar ratio near
10 3/1.

- Last but not least, it is envisaged by the invention to use transdermal carriers, typically in the form of barrier penetrating extended surface aggregates, to deliver NSAIDs below the skin and into the underlying muscle tissue or the
15 adjacent joints.

- A list of potential ingredients that can be used for preparing pharmaceutical formulations according to the present invention is given in Cosmetic Ingredient Review (CIR Compendium), which is regularly
20 published in Washington, DC, and in appropriate Food and Drug Administration or other national regulatory agency publications, including the list of GRAS (Generally Recognised As Safe) compounds.

- It is furthermore an explicit aim of the document, to teach the use of amphipat
25 combinations, as described herein, as drug carriers, drug depots, or for other kind of medicinal or biological application. For the purpose the required extended surfaces are advantageously provided in the form of membranes formed by the at least one first substance, the at least one second and the at least one third substance, which together surround miniature droplets. The
30 substance with a biological activity, such as a drug, is then mainly associated

- 86 -

with said droplets at the surface or else is mainly incorporated into the droplet to be carried by the droplet to the place where the biologically active substance is supposed to act.

- 5 Relatively detailed recommendations for preparing compositions, as advocated in this application, are given in EP 0 475 160 and US 6 165 500, which are herewith included by reference. When filtration is used to prepare aggregate suspensions, filter material with pore diameters between 0.01 μm and 0.1 μm , more preferably with pore diameters between 0.02 μm and 0.3
10 μm and even more advisable with pore diameters between 0.05 μm and 0.15 μm are used for homogenisation.

- The present patent application moreover teaches suitable methods for preparing combinations such that solve the outlined problems by providing
15 suitable formulations of biologically, cosmetically and/or pharmaceutically active agents, comprising the steps of: a) selecting the at least one first and the at least one second substance which together form extended surfaces, when in contact with said liquid suspending medium, such that said extended surfaces formed by the at least one first and the at least one second
20 substance are more adaptable than the at least one first substance alone and the surfaces formed by the at least one second substance alone are not extended; alternatively; b) selecting the at least one first and the at least one third substance which together form extended surfaces, when in contact with said medium, such that said extended surfaces formed by the at least one
25 first and the at least one third substance are more adaptable than the at least one first substance alone and the surfaces formed by the at least one third substance alone are not extended, if this substance self-aggregates; and c) generating said combination of at least one first, at least one second, and at least one third substance, such that the surface of resulting at least three
30 component combination is even more adaptable than the surface prepared

- 87 -

from at least one first and one second substance alone or of the surfaces formed by the at least one first and one third substance alone, bringing the combination of at least two or all three said substances into suspension by means of controlled mechanical fragmentation, preferably in the presence of
5 or before being mixed with the at least one third substance, such that said third substance is incorporated at least partly in said extended surface formed by controlled mechanical fragmentation to obtain final preparation.

It is particularly preferred to use filtration, pressure change or mechanical
10 homogenisation, or else shaking, stirring, or mixing as said means of controlled mechanical fragmentation. The desirable intermediary or final characteristics of the liquid medium used to prepare aggregate suspension are defined in previous paragraphs of this section.

15 The present patent application furthermore teaches methods based on use of said at least quaternary mixtures containing at least one active agent selected from the group comprising anti-diabetic agents, growth factors, immunomodulators, enzymes, recognition molecules, adrenocorticostatics, adrenolitics, androgens, antiandrogens, antiparasitics, anabolics,
20 anaesthetics, analgesics, analeptics, antiallergics, antiarrhythmics, antiarterosclerotics, antiasthmatics, bronchospasmolytics, antibiotics, antidrepressiva, antipsychotics, antidots, antiemetics, antiepileptics, antifibrinolytics, anticonvulsiva, anticholinergics, enzyme, coenzymes or corresponding inhibitors, antihistaminics, antihypertonics, biological inhibitors
25 of drug activity, antihypotonics, anticoagulants, antimycotics, antimyasthenics, agents against Morbus Parkinson or Morbus Alzheimer, antiphlogistics, antipyretics, antirheumatics, antiseptics, respiratory analeptics or respiratory stimulants, broncholytics, cardiotonics, chemotherapeutics, coronary dilators, cytostatics, diuretics, ganglium-
30 blockers, glucocorticoids, antiflow agents, haemostatics, hypnotics,

- 88 -

immunologically active substances, contraceptives, anti-migraine agents, mineralo-corticoids, morphine-antagonists, muscle relaxants, narcotics, neurotherapeutics, neuroleptics, neurotransmitters or their antagonists, peptides, peptide derivatives, ophthalmics, sympaticomimetics or
5 sympatricolytics, para-sympaticomimetics or para-sympatricolytics, anti-psoriasis drugs, neurodermitis drugs, mydriatics, psychostimulants, rhinologics, sleep-inducing agents or their antagonists, sedating agents, spasmolytics, tuberculostatics, urologics, vasoconstrictors or vasodilators, virustatics, wound-healing substances, or a combination of aforesaid agents.

10

Aforesaid method can rely on either using the recommended at least three amphiphilic substances as such, or else dissolved in a physiologically compatible polar fluid, comprising water or water-miscible fluids, or in solvation-mediating agent, together with a polar solution. Use of co-solvents
15 is also possible.

A preferred, particularly practical method for preparing said aggregate formulations contains at least one surfactant or surfactant-like amphipat, which destabilises bilayer membrane, and at least one more membrane
20 destabilising, biologically active ingredient or an additional surfactant in said polar solution.

In the case of need, the method can include the formation of said surfaces induced by addition of one or more formulation or aggregate components into
25 a fluid phase, e.g. by using evaporation from a reverse phase, injection or dialysis, or even by additional mechanical stress.

Furthermore, it may be preferred to use preparation method in which the formation of said surfaces is induced by filtration, the filtering material having
30 pores between 0.01 μm and 0.8 μm wide. The choice of most convenient or

favourable pore diameter depends on the desired final aggregate dimensions and also on the anticipated or achieved suspension flux through a filter. Higher flux rates produce stronger shear and relatively smaller final vesicle diameter, suspension viscosity also being important.

5

When filtration is used to manufacture aggregate suspensions, it may be convenient to use several filters sequentially or in parallel. In the former case, pore diameters in different filters can vary in diameter.

- 10 An preferred advantageous method for preparing suspensions according to the present invention is such that ensures said agents and carriers to associate, at least partly, after the formation of said extended surfaces.

- 15 For better convenience, said extended surfaces, with which agent molecules are made to associate, may be prepared just before the application of the formulation. If desired, and possible, this can be done from a suitable concentrate or a lyophilisate.

- 20 It is practically convenient to use a single container comprising the selected pharmaceutical composition based on the combination of substances as described in previous text. It is also convenient to make said container a part of a package.

- 25 The present patent application moreover teaches a method for generating a therapeutic effect on a warm blood creature by applying a suitably selected pharmaceutical composition onto or into a leaving creature's body, whereby the selection of a suitable combination of substances is made according to the claims of this document.

Special application of the method described herein is to choose such administration volume that ensures control over the applied medicament dose and the outcome of therapeutic application.

- 5 It may be preferred, and practically valuable, to load a suspension of drug-free aggregates with the drug, via association, during the day prior to an administration, preferably within 360 min, more preferably within 60 min and most preferably within 30 min time window before the administration of resulting formulation in or on the body.

10

The method of treatment done according to the present invention typically involves administration of at least one dose of the pharmaceutical composition with therapeutic activity on or in a warm blood animal.

- 15 Last but not least, the present invention teaches a method for finding suitable compositions, as described herein. This method comprising the steps of: a) determining the flux of aggregates in a suspension associated with a drug through pores in a well-defined barrier, or various barriers, as a function of the driving force or the driving pressure, which acts across the barrier; b)
20 describing the data within the framework of a suitable model such that fits the characteristic flux vs. pressure or penetrability vs. pressure curve; c) to deduce the characteristic system parameters, such as p^* and P_{max} , in particular; d) employing said parameters to optimise or characterise the formulation for application. Eq. (*) is recommended as, and is claimed herein
25 to be, particularly suitable for describing and analysing such data.

Practical Examples

The following examples illustrate the invention without setting or delineating its limits. All temperatures are in degree Celsius. Carrier diameters are in
5 nanometers, pressures in Pascal (Pa) and other units correspond to standard SI system. Ratios and percentages are given in moles, unless otherwise stated.

All measurements were done at room temperature, except when specified
10 otherwise. For aggregate adaptability / barrier transport resistance measurements the test temperature was constant to within plus/minus 2 degrees. For aggregate size measurements the temperature accuracy was plus/minus 0.1 degree. The pH value of the bulk suspension was determined with a commercial (gel) electrode. All substances were used as received and
15 were of p.a. quality, unless stated otherwise. Molar masses were taken to be identical to the published reference data.

Suspension viscosity was measured with a rotation viscosimeter, typically at
20 room temperature and using 20 RPM, which corresponded to 150 1/s.

Determination of Barrier Transport Resistance and aggregate Adaptability.
Barrier resistance to the transport of test vesicle suspension in earlier patent applications by the same applicant was called "permeation" resistance. In
this document, more precise term "penetration" resistance is used to stress
25 the fact that vesicles do not diffuse (=permeate) through but rather penetrate barriers.

In first approximation one relies on simple experimental method (SEM) and takes barrier transport resistance (in arbitrary units) to be proportional to the
30 pressure (in arbitrary units) needed to drive a suspension of relatively large

vesicles through a 0.2 micrometer filter with good efficacy. (In our experience, a porous filter acts as a permeability barrier when the average pore diameter is at least 40% to 50%, for the vesicles bigger and smaller than 150 nm, respectively, and more preferably is at least 100% smaller than the average vesicle diameter in the tested suspension.) Barrier transport resistance is then given in relative units of 1 to 10 elsewhere (in EP 0 475 160 and USP 6 165 500) and in this document whenever reference is made to a 0.2 micrometer filter. Barrier penetrability, which in older publications is called permeability, is identified with inverse barrier resistance value. Aggregate adaptability is a direct function of the former value, as is explained e.g. in Critical Reviews in Therapeutic Drug Carrier Systems 13:257-388 (1996) or in Adv. Drug Delivery Rev. 18:349-378 (1996).

Use of relative penetrability and barrier resistance values is also convenient. These values are given by the ratio of the penetrability/permeability or of the corresponding barrier resistance values measured with a given suspension and its supporting medium (e.g. water), e.g.: (relative) Penetrability $\eta P_{rel} = P_{suspension} / P_{medium}$. Similar use of the trans-barrier flux data, measured with constant driving pressure, provides more direct but still relative measure of barrier penetrability/permeability for different formulations. Theoretical explanation for such comparisons and calculations is given in Critical Reviews in Therapeutic Drug Carrier Systems 13:257-388 (1996).

To get an absolute Barrier Transport Resistance or Penetrability data, and to interpret these values in molecular terms, an improved analytical method is needed, which is described in brief in Definitions sections (see especially e.q. (*)). To get absolute penetrability - and thus aggregate adaptability - data, transbarrier flux is first measured serially. (This can be done as is described in this document or in Biochim. Biophys. Acta 1368: 201-215 (1998).) Barrier resistance / penetrability value for the test suspension is then calculated from

the flux vs. pressure data using e.q. (*), following the description given in previous sections. From calculated resistance / penetrability value, a convenient parameter that describes the adaptability of mixed aggregates is deduced, e.g. by assuming: $a_a = 1/p^*$.

5

Aggregate adaptability is thus identified with the inverse pressure difference needed to attain a predefined, practically relevant fraction of maximum achievable flux-pressure ratio; using 50-60% maximum penetrability criterion gives reasonable results. Specifically, all p^* values given in this document correspond to 57% of P_{max} -value. If the maximum penetrability for a given suspension-barrier combination cannot be measured, the penetrability of a barrier to the medium in which the tested aggregates are suspended is used as surrogate: $P_{max} = f \times \text{Suspending medium flux} / \text{Driving pressure}$. Proportionality factor is then typically taken to be up to 3-times (and more often up to 2-times) smaller than 57%, to allow for trivial friction effects.

Exemplary results are given in figures 4 and 5. The latter figure also graphically illustrates the meaning of parameters " p^* " (in pressure units, and proportional to the barrier transport resistance) and "Maximum penetrability" (= P_{max} ; in flux per pressure units, and indicative of barrier porosity).

Aggregate size (diameter) determination. The average aggregate (most often vesicle) diameter was measured with the dynamic light scattering (for a few samples with a Malvern Zeta-Sizer instrument and for the majority of samples with the instrument with an ALV 5000 correlator. Cumulant analysis method and an implementation of software package "Contin" were used for analysing the correlation curves obtained with Zeta-Sizer. To analyse the ALV measurements the software delivered by the manufacturer (cumulant analysis or "Contin") was employed.

30

Examples 1-120:Composition:

5	37.74 - 84.5 mg	Phosphatidylcholine from soy-bean (SPC, ~ 85% purity, <i>MFC</i>) introduced as an ethanolic solution SPC/EtOH = 1/1 V/V and containing approx. 10% charged phospholipid (presumably anionic phosphatidylglycerol)
10	187-34.9 mg	Polysorbate (Tween 80, pharmaceutical grade; <i>MDC</i> ₁)
	5.6 - 20 rel. mol-%	Sodium dodecylsulphate (SDS, p.a.; <i>MDC</i> ₂) replacing phospholipid to the given amount
	ad 1 ml	Isotonic phosphate buffer (pH = 7.2)

15

Objective: to test the synergism between membrane destabilising, and thus aggregate adaptability increasing, activity of two different surfactants, used in a combination with a lipid, as the basic membrane forming system component.

20

Suspension preparation. To prepare a series with changing lipid/surfactant ratio in the range 1/1 to 9/1, the necessary amounts of phospholipid and surfactant are pipetted into buffer to yield 10% lipid suspensions. These are first stirred at room temperature for 5 days and then pre-filtered through a 0.8 micrometer polycarbonate filter to narrow down the starting aggregate diameter. The average vesicle diameter ($2r_{ves}$) is determined and confirmed to exceed at least 2-fold the nominal diameter of pores in the test filter ($2r_{pore}$), which is approximately 0.2 micrometer. This is done with the dynamic light scattering e.g. by using a Malvern Zeta-Sizer instrument.

30

Transport (pore penetration) capability. Transport resistance is equated with the volume of test suspension that does not pass through a 0.2 micrometer filter in a sterile holder. (A ready-to-use, commercially available "blue" filter unit of Sartorius (Göttingen, Germany) is used for the test.) This reveals that

5 transport resistance decreases with increasing Tween content when the relative SPC content is lower than 6/1 (SPC/Tw); see also examples 40-49 in EP 0 475 160. The trend is enhanced by the presence of sodium dodecylsulphate in the mixed lipid aggregates. The latter shifts the minimum amount of Tween needed to cross the semi-permeable barrier to increasingly

10 lower relative concentration values.

For example, when 12 mol-% of SPC in the mixed amphipat aggregates is replaced by SDS, the suspension can be pushed through a barrier with 0.2 micrometer pores practically without transport resistance even when the

15 relative SPC/Tween concentration is as low as 15/1. Increasing SDS content further does not improve the situation, as measured in this test series. In contrast, reducing SDS content to and below 10 mol-% relative to SPC shows a clear deterioration of penetration ability of the resulting quaternary suspension. Rather low transport resistance is now measured for

20 SPC/Tween 7/1 (in case of 10 mol-% SDS concentration) and for SPC/Tween 4/1, when SDS concentration is between approx. 2 mol-% and 5 mol-%, as can be seen from figure 6. In contrast, maximum barrier resistance value of 10 is found for the suspensions without SDS and/or with little Tween and SDS, the properties of which approach those of plain, single

25 component liposomes, which also have characteristic resistance value of 10.

Post-test determination of vesicle diameter confirms that vesicles are still at least 1.3-times greater than the nominal pore diameter.

Examples 121-129:Composition:

	14.2 mg	Polysorbate (Tween 80)
5	85.8 mg	Phosphatidylcholine from soy-bean (SPC), as with examples 1-120
	0 - 17.5 rel. mol-%	Sodium dodecylsulphate (SDS), relative to SPC and replacing phospholipid to the given amount
	ad 1 ml	Isotonic phosphate buffer (pH = 7.2)

10

Objective: as with examples 1-120, to test the synergism of different surfactant action on extended surface aggregate properties.

15 Suspension preparation. The method used to prepare vesicle suspension was the same as in examples 1-120. The only notable difference between both test series was the somewhat greater average diameter and polydispersity of the vesicles used in examples 121-129.

20 Transport ability (pore penetration capability and adaptability) of aggregate suspension . To characterise the resistance of semi-permeable barrier to suspension flux (= transbarrier flux), the same method as in examples 1-120 was used. The resistance was measured as a function of relative SDS concentration in bilayer, to determine minimum amount of this latter surfactant that is needed to maximise suspension flux across the barrier and

25 minimises the barrier transport resistance value. Experimental data suggest that the threshold limit is around 6 mol-%, with some uncertainty in the 2-6 mol% region. This is consistent with the results of first test series (examples 1-120) except in that the measured resistance values are now somewhat higher. This is explicable by different starting vesicle diameter and

30 polydispersity. The results are given in following table.

Table 1: The effect of SDS, as the second surfactant (MDC_2) in addition to Tween 80 (MDC_1 ; 10 mol-% rel. to SPC), on the resistance of mixed lipid membranes containing phosphatidylcholine (SPC; MFC), as the basic building block, to the passage through a semi-permeable barrier with pores, which were at least ~50% smaller than the average aggregate diameter.

SDS/SPC [mol/mol]	Barrier transport resistance [rel. units, as defined in SEM]
0/100, reference Tween Tfs	10
2/98	4
4/96	10
6/94	1.88
8/92	1.75
10/90	1.50
25/175	1.12
15/85	0.75
35/165	0.44

10

Examples 130-131:Composition:

52.1 mg	Phosphatidylcholine from soy-bean (SPC), actual amount = 52.2 mg - Na Chol amount in mg
45.2 mg	Polysorbate (Tween 80)
5, 10, 15 mol-%	Sodium cholate = Na Chol (relative to SPC in the suspension)
ad 1 ml	Isotonic phosphate buffer (pH = 7.2)

15

Objective: as with examples 1-120, but using a different charged surfactant (cholate instead of SDS).

- 5 Suspension preparation. The starting suspension was prepared as in previous examples. However, to make vesicles in the test formulation more uniform before actual measurements, the starting suspension was pre-filtered through 80 nm pore filters. This yielded vesicles with approx. 120 nm diameter, as determined with the dynamic light scattering using ALV 5000
10 correlator and a personal computer.

- Vesicle transport ability (pore penetration capability / adaptability). The actual transport test was done with relatively narrow pore (30 nm) filters, using different pressures applied on the filter to characterise the penetrability
15 of such semi-permeable filter to the test suspension. This revealed fairly comparable penetration ability for the vesicles with 10 mol-% and 15 mol-% cholate, exceeding the pore penetration ability, and thus the adaptability, of the vesicles with merely 5 mol-% of cholate as the third membrane destabilising component (cf. figure 3). These results indicate that
20 incorporation of the second surfactant into mixed lipid bilayers does not increase membrane adaptability proportionally, as one would expect on the basis of model results shown in figure 7.

Examples 132-138:

25

Composition:

- | | |
|-------------|---|
| See Table 2 | Phosphatidylcholine from soy-bean (SPC) |
| See Table 2 | Ketoprofen, sodium (KT); |
| See Table 2 | Tween 80, see Table 2 |
| 30 Ad 1 ml | Phosphate buffer (pH = 7.2) |

Objective: to test the synergistic effect of a membrane destabilising drug (KT) combined with a surfactant (Tween 80) in a lipid (SPC) suspension in terms of mixed aggregate adaptability and relative capability to cross
5 semipermeable barriers.

Test suspension preparation. The stated phospholipid and drug amounts were brought into suspension using mechanical homogenisation. That resulting average aggregate diameter was around 100 nm.

10 Vesicle transport ability (pore penetration capability / adaptability). The efflux of the test suspension from a vessel pressurised with nitrogen gas was measured as a function of the time to determine the pressure dependency of material transport through the 20 nm pore filter in front of an opening in the measuring vessel. From the measured flux data, the effective "barrier
15 penetrability", which defines the adaptability of the tested mixed amphipat vesicles, was calculated as is described in the main text body. The measured curves were also analysed in terms of the pressure p^* , needed to achieve 57% of maximum possible suspension flux/pressure ratio. The result of the test series indicate that both ketoprofen and Tween can act as a
20 membrane destabilising component. Consequently, either of these two system ingredients improves the ability of test suspension to penetrate barriers compared with simple phosphatidylcholine, reference liposomes in a suspension without KT or Tween 80. When a combination of said membrane destabilising components is used, extended surface aggregate adaptability
25 is increased to the value measured with proper non-ionic Tween-based Transfersome® suspension, with surfactant concentration much higher than that used in the quaternary mixture. Data given in Table 2 justify the conclusion. They are also compared with those pertainint to simple buffer fluid (Ref. fluid) in which the mixed SPC/KT/Tween vesicles were
30 suspended.

Table 2: Experimental and fit results for the pore penetration experiments done with various quaternary suspensions of a phospholipid (SPC; *MFC*), a drug (KT; *MDC*₁), and Tween 80 (*MDC*₂) co-suspended in a buffer ; TL = total lipid

Tween 80 [mol% of SPC]	Ketoprofen [mol% of TL]	p^* [MPa]	P_{max} [10^{-11} m Pa ⁻¹ ·sec ⁻¹]	Adaptability a_a , [MPa ⁻¹]
0 (Liposomes)	0	> 3	Not measurable	(< 0.3)
0	25	2.41 ± 0.15	Not measurable	0.415
0	33	1.66 ± 0.07	345 ± 37	0.602
10	33	0.25 ± 0.03	230 ± 17	4.000
50	0	0.20 ± 0.01	227 ± 3	5.000
0 (=Ref. Fluid)	0	Not applicable	613 ± 15	Not applicable

Examples 139-142:

10

Composition:

75.0 mg

Phosphatidylcholine from soy-bean (SPC),
used as a saturated ethanolic solution
SPC amount = 75 mg - Brij 98 content given in

15

Table 3

25.0 mg

Ketoprofen, sodium (KT)

See Table 3

Brij 98

Ad 1 ml

Phosphate buffer (pH = 7.2)

20

Objective: to test adaptability / pore penetrability supporting activity of a different surfactant (Brij) combined with a membrane destabilising drug (KT) in lipid (SPC) extended surface aggregates.

Suspension preparation was essentially the same as in examples 132-135.

- Vesicle transport ability (pore penetration capability / adaptability). In order to
- 5 test whether or not the increased adaptability of SPC/KT ternary suspensions is a unique feature of Tween, as the fourth component, the effect of another surfactant was investigated. In order to avoid undesired electrostatic interactions between the anionic KT and such additive, the uncharged Brij 98 (oleoyl-chain, 20 oxyethylene units per molecule) was chosen. The
- 10 penetrability of resulting SPC/KT/Brij 3/1/0-0.323 w/w/w mixtures was finally calculated using eq. (*).

The results for similar series measured with Brij 98 are given in Table 3.

- 15 **Table 3:** Fit results, based on e.q. (*), for the transbarrier flux of suspensions containing a lipid (SPC; *MFC*), a drug (KT; *MDC₁*) and Brij 98 (*MDC₂*) in different relative concentrations, SPC and Brij together representing the total lipid (TL)

Brij 98 [mol% of SPC]	KT [mol% of TL]	p^* [MPa]	P_{max} [$10^{-11} \text{ m Pa}^{-1} \cdot \text{sec}^{-1}$]	Adaptability a_a , [MPa $^{-1}$]
0	33	1.66 ± 0.07	345 ± 37	0.602
2.5	33	0.56 ± 0.07	266 ± 28	1.786
5.0	33	0.29 ± 0.07	191 ± 30	3.448
7.5	33	0.32 ± 0.06	171 ± 21	3.125

20

[§]The quoted error only accounts for analytical and not for experimental data uncertainty, which for example 16 exceeds 80%

Examples 143-146:Composition:

	100 mg	Total lipid (TL, including SPC and Tween 80)
5	See Table 4	Phosphatidylcholine from soy-bean (SPC)
	See Table 4	Tween 80
	See Table 4	Diclofenac
	See Table 4	Ethanol (EtOH)
	5.25	Benzyl alcohol
10	Ad 1 g	154 mM Phosphate buffer, pH = 7.2

Objective: to test the effects of a surfactant (Tween 80) and a drug (diclofenac), as two membrane destabilising amphipats, and of a short-chain alcohol (ethanol) as an additional - and potentially the second membrane destabilising amphipat.

Vesicle preparation was done essentially as described in example 8 of WO 98/17255, but a more modern version of barrier penetration assay was used to assess vesicle aggregate adaptability. For historic comparison, vesicles with a similar overall composition but lacking ethanol were tested (cf. examples . The results are given in Figure 8 and in Table 4.

Due to the limited measuring range of pore penetration assay, it was only possible to obtain a rough estimate for the adaptability of extended surface aggregates tested in this test series. The estimated p^* -value of the preparations containing ethanol were lowered to ~1.6 MPa from ~4.8 MPa measured in the absence of this alcohol. (It must be kept in mind, however, that experimental variability in these tests was at least 50%, as the standard deviations given in Table 4 only stem from the fit routine.) The direction of the

change is reasonable, but the calculated absolute difference in p^* is not significant.

Table 4: Results of driving pressure and aggregate adaptability analysis for the test.

Ex	Tween 80 [mol% of SPC]	EtOH [w-%]	Diclofenac [w-% of TL]	p^* [MPa]	P_{\max} [10^{-11} m Pa $^{-1}$ sec $^{-1}$]	Adaptability a_a , [MPa $^{-1}$]
143	0	0	10	(4.8 \pm 1.6)	Not measurable	0.208
144	0	9	10	2.4 \pm 0.04	402 \pm 17	0.417
145	0	9	20			
146	10	9	10			

^sThe quoted error only accounts for analytical and not for experimental data uncertainty, which for example 16 exceeds 80%.

Data given in Figure 8 and in Table 4 imply that ethanol makes the tested lipid aggregates more adaptable. The effect is much smaller, however, than in case of using a surfactant, such as Tween 80 (see Table 2).

Simple use of a membrane destabilising drug (diclofenac) and of a short-chain alcohol, as membrane softening agents disclosed in the prior art, thus only produces extended surface aggregates with an adaptability significantly inferior to that of the formulations disclosed in the present invention.

Specifically, the formulation described in Example 8 of WO 98/17255 is capable of crossing semipermeable barriers with narrow pores, but leaves space for further improvement. Ethanol containing, diclofenac loaded vesicles, indeed, are more adaptable than the ethanol-free vesicles. However, even the former vesicles have a much higher p^* value, and therefore are far less adaptable, than the ternary mixtures of

phosphatidylcholine, a non-ionic surfactant (Tween 80) and ketoprofen described in Table 2; the beneficial effect of a surfactant-like membrane destabilising component, such as Tween 80, is directly reflected in the lower p^* value and/or in a higher flux of the modified formulation through a barrier.

- 5 This conclusion is practically unaffected if the latter formulation contains ethanol.

- 10 It therefore stands to reason that at least two membrane destabilising components should be present in an aggregate with extended surface adequate quantities to maximise the adaptability of extended surface aggregates. Mere use of a lipid, ethanol and a drug, as is disclosed in the prior art, is insufficient for reaching the goal.

15 **Examples 147-150**

Composition:

- | | |
|--------------|--|
| 80.0-71.4 mg | Phosphatidylcholine from soy-bean (SPC) |
| 20-28.6 mg | Ketoprofen, sodium (KT), replacing SPC |
| 20 | in the suspension to achieve constant amphipat amount |
| ad 1 ml | Phosphate buffer, pH = 7.2,
if necessary readjusted with NaOH |

- 25 Objective: to demonstrate that ketoprofen, an NSAID, acts as membrane destabilising component and can render mixed amphipat aggregates with extended surface adaptable enough to penetrate narrow pores.

- 30 Test suspension preparation. The stated phospholipid and drug amounts were brought into suspension using mechanical homogenisation. That

resulting average aggregate diameter was around 100 nm. For reference, a comparable suspension containing SPC and sodium cholate in 3.75/1 mol/mol ratio was used.

5 Vesicle transport ability (pore penetration capability / adaptability). The efflux of the test suspension from a vessel pressurised with nitrogen gas was measured as a function of the time to determine the pressure dependency of material transport through the 20 nm pore filter in front of an opening in the measuring vessel. From the measured flux data, the effective "barrier
10 penetrability", which defines the adaptability of the tested mixed amphipat vesicles, was calculated as is described in the main text body. The measured curves were also analysed in terms of the pressure p^* , needed to achieve 57% of maximum possible suspension flux/pressure ratio. The calculated p^* -value decreased from 2.41 ± 0.15 MPa (mean value \pm standard
15 error) through 1.66 ± 0.07 MPa to 1.36 ± 0.10 MPa with increasing drug concentration. This is indicative of membrane destabilising activity of the drug, which arguably promotes bilayer flexibility and permeability. More detailed information is given in Table 5, which reveals essentially identical p^* values for the SPC/KT 3/1 mol/mol mixture and for the reference anionic
20 Transfersome® suspension. To deduce vesicle adaptability from p^* -value, contribution from suspension viscosity effects must be included or must be known to be negligible. This is not an issue, however, as long as one can make comparisons with suitable reference formulation(s), as is done in the following table by inclusion of last line.

25

In this test series, as in all other practical examples reported herein, the final aggregate diameter after narrow pore crossing was at least 300%, and typically was more than 400% greater than the pore diameter, the final to starting aggregate diameter ratio being typically > 0.70 , implying
30 fragmentation of less than 30%, and more often merely 10-20%.

Table 5: Fit results, based on eq. (*) for the barrier penetrability (flux/pressure ratio) experiments done with the suspensions characterised by different lipid/drug, SPC/KT ratios

SPC/KT [mole/mole]	p^* [MPa]	P_{max} [10^{-11} m Pa $^{-1}$ ·sec $^{-1}$]	Adaptability a_a , [MPa $^{-1}$]
10/0	~3	Not measurable	~0.3
4/1	2.41 ± 0.15^s	Not measurable	0.415
3/1	1.66 ± 0.07^s	-	0.602
2.5/1	1.36 ± 0.10^s	345 ± 37	0.735
Reference anionic Tfs ss	1.76 ± 0.13^s	318 ± 39	0.568

5 ^sThe quoted error only accounts for analytical and not for experimental data uncertainty, the latter often amounting to 20-30%. ^{ss} These Tfs vesicles were prepared from an SPC/Na cholate 3.75/1 mol/mol mixture.

10 Graphic representation of the results of these experiments is given in Figure 9.

Examples 151-153:

15 Composition:

75.0, 75.0, 37.7 mg	Phosphatidylcholine from soy-bean (SPC)
25.0, 25.0, 0.0 mg	Ketoprofen, sodium (KT)
0.0, 25.4, 62.3 mg	Tween 80
0.0, 0.0, 37.7 mg	Ethanol
20 ad 1 ml	Phosphate buffer (pH = 7.2)

Objective: to test the synergistic effect of the second and first membrane destabilising amphipat (Tween 80, ketoprofen, respectively) in terms of an extended surface aggregate adaptability.

- 5 Suspension preparation was essentially the same as with examples 147-150.

Vesicle transport ability (pore penetration capability / adaptability).

Transbarrier flux of the test suspension containing 5 mol-% Tween is much higher than for the formulation that contains merely phospholipid (as the
10 basic amphipat) and ketoprofen (as the surface active, membrane destabilising, surfactant-like amphipat) components. This is clearly seen from Figure 10, which illustrates pressure dependence of said suspension flux divided by driving pressure.

15

Examples 154-158:

Composition of aggregates:

75.0 mg	Phosphatidylcholine from soy-bean (SPC),
20	the actual value is: 75 mg - Tween 80 amount in
mg	
25.0 mg	Ketoprofen, sodium (KT)
see the following table	Tween 80
ad 1 ml	Phosphate buffer (pH = 7.2)

25

Reference buffer: Phosphate buffer (pH = 7.2)

Objective: to study the effect of relative concentration of a surfactant, as the second membrane destabilising amphipat, on adaptability of extended
30 surface mixed amphipat aggregates.

Suspension preparation: as with examples 147-150.

Vesicle transport ability (pore penetration capability / adaptability) data, as measured in this test series, confirm and expand the findings obtained with examples 147-150. Tween acting as the second membrane destabilising component improves the ability of test suspension to penetrate barriers even when this surfactant is present in the quaternary mixture merely in small amount, as long as relative concentration of Tween is at least approximately 2.5 mol-%, and even better 5 mol-%. Data given in Table 6 justify the conclusion. They are compared with the reference non-ionic Tween-based Transfersome® formulation (Reference Tfs) and with the buffer fluid (Reference fluid) in which mixed amphoteric vesicles were suspended.

The suspension viscosity for example 157 was around 730 mPa s at 20 RPM

Table 6: Fit results for the pore penetration experiments done with various quaternary suspensions of a phospholipid (SPC; stable membranes forming component), a drug (KT; 1st membrane destabilising component), and Tween 80 (2nd membrane destabilising component) co-suspended in a buffer at different relative concentrations of Tween 80.

Nr	Tween 80 content [mol% of SPC]	p^* [MPa]	P_{max} [10^{-11} m Pa $^{-1}$ ·sec $^{-1}$]	Adaptability a_a , [MPa $^{-1}$]
	0	1.66 ± 0.07	345 ± 37	0.602
154	1.25	0.51 ± 0.05	293 ± 23	1.961
155	2.5	0.50 ± 0.04	339 ± 26	2.000
156	5	0.23 ± 0.03	215 ± 19	4.348
157	7.5	0.22 ± 0.02	213 ± 14	4.545
158	Reference Tfs (Tween)	0.20 ± 0.01	227 ± 3	5.000
	Reference fluid (buffer)	Not applicable	613 ± 15	Not applicable

^sThe quoted error only accounts for analytical and not for experimental data uncertainty, the latter often amounting to 20-30%.

Reference Tfs vesicles were prepared from an equimolar (50/50 mol/mol)

5 SPC/Tween 80 mixture.

Examples 159-160:

10 Composition:

43.65 mg Phosphatidylcholine from soybean (+95% = PC)
72.00 mg Tween 80
34.35 mg Ketoprofen
6.08 mg Sodium hydroxide
15 5.25 mg Benzyl alcohol
36.51 mg Ethanol 96%
ad 1 g 154 mM phosphate buffer, pH = 7.4

Objective: The confirm novelty of the formulations described in this
20 application in general.

For the purpose, the closest examples given in previous relevant patents (applications) were reproduced combining all the potentially relevant explicit teachings from such previous art documents. Experimental procedures were selected correspondingly, except for the usage of more modern analytical
5 methods.

Suspension preparation: On the one hand, the concentration of the surfactant Tween 80, which acts as a membrane destabilising component, was used at the level suggested by examples 40-49 of USP 6 165 500 (or of the
10 equivalent EP 0475 160 A1). An equimolar mixture of phosphatidylcholine and Tween 80 was chosen, as the adaptability of such mixture, expressed in terms of its inverse value which is proportional to the tested barrier resistance, for such mixture approaches zero. On the other hand, the partially ionised ketoprofen, which binds to lipid bilayers and makes such
15 membranes more flexible, was used as the second membrane destabilising component. Such a drug usage is taught explicitly in WO 98/17255 for two other NSAIDs: diclofenac and ibuprofen, e.g. in Practical Examples 8-17 and 18-25.

20 The tested membrane composition thus corresponded to the optimum SPC/KT ratio suggested in Table 6 for the SPC/KT/Tween 80 mixtures with an increased molar SPC/Tween 80 ratio. The weight percent of ketoprofen, relative to the total lipid concentration, was thus around 3/1 and hence similar to that taught in said examples in WO 98/17255 for diclofenac, which cover
25 molar ratios 4/1 to 1/4. Also in accord with WO 98/17255, an isotonic phosphate buffer was used to suspend the resulting mixed lipid vesicles.

Hydration of the components mixed in given proportions produced a clear, yellowish fluid. This is indicative of micellar suspension and implies that the
30 tested mixed lipid aggregates are colloiddally not stable. Determination of the

- 111 -

average diameter of aggregates in such suspension with the dynamic light scattering confirmed the conclusion (mean particle diameter approx. 22 nm, which is incompatible with existence of vesicles).

- 5 Diluting the preparation with the corresponding buffer from 15% total lipid to 10% total lipid, making essentially the same observation further corroborated the result. Based on the existing information about phosphatidylcholine solubilisation by Tween 80, even a reduction of relative surfactant concentration by a factor of 2, thus creating a SPC/Tween 80 2/1 mol/mol
10 mixture loaded with approx. 30 mol-% ketoprofen, still would yield unstable aggregates.

- Addition of Tween 80 much beyond the rather low relative molar concentration proposed in example 158 thus destabilises the three
15 component lipid aggregates to the point of solubilisation, or at close to this point. Compositions originating from the combination of relevant experimental teachings in patents WO 98/17255 and EP 0475 160 A1 therefore do not fulfil the required stability criterion for the extended surface aggregates and consequently do not represent prior art to present
20 application.

Comparative Examples 161-162:

25 Composition:

- | | |
|-----------------|--|
| 66.71 mg | Soybean-phosphatidylcholine |
| 11.00 mg | Tween 80 |
| 22.21 mg | Ketoprofen |
| 0.00 / 66.71 mg | Ethanol (EtOH; for examples 16 and 17, respectively) |
| 30 11.56 mg | NaOH (30%) |

- 112 -

	0.50 mg	Na metabisulphite
	1.00 mg	Disodium edetate (EDTA)
	0.20 mg	Butylhydroxytoluene (BHT)
	1.46 mg	Methylparabene
5	1.00 mg	Linalool
	5.25 mg	Benzyl alcohol
	ad 1 g	7.8 mM Phosphate buffer, pH = 7.2

- Objectives: First, to check membrane destabilising and aggregate
- 10 adaptability increasing effect of ethanol was in the range of concentrations described in previous inventions (see USP 6 165 500 or the equivalent EP 0475 160 A1). The results, given in Table 7, confirm that the adaptability of the aggregates proposed in prior art is far inferior to that of the newly proposed formulations.
- 15
- Second, to test the effect of system stabilisers (Na metabisulphite; EDTA; BHT, benzyl alcohol) on essential characteristics of extended surface aggregates. The results confirmed the that the key system parameters, which determine the suspension ability to cross semipermeable barriers, i.e.
- 20 pressure p^* and aggregate adaptability, are not inacceptably affected by such additives.

- Suspension preparation. Vesicular intermediate preparation with 17.14% total lipid containing no ethanol and ketoprofen in identical concentration as in
- 25 Example 157 was mixed with the SPC mass equivalent of ethanol. This was done to match as closely as possible the examples 8-17 given in WO 98/17255. To meet the needs of pharmaceutical formulations as well, several suspension stabilising agents (EDTA, BHA, methylparabene, and benzyl alcohol) were included in the formulation. Characterisation was done
- 30 as with examples 147-150.

Table 7: Results of driving pressure and aggregate adaptability analysis for examples 15 and 16.

Formulation	p^* [MPa]	P_{\max} [10^{-8} kg / (m ² ·s·Pa)]	Adaptability a_a , [MPa ⁻¹]
Example 161 (no EtOH)	0.233 ± 0.013^s	216.5 ± 7.4	4.292
Example 162 (with EtOH)	0.133 ± 0.006^s	254.3 ± 9.7	7.519

^sThe quoted error only accounts for analytical and not for experimental data

5 uncertainty.

Specifically, the pressure required to drive vesicles through narrow pores, p^* , was found to decrease in the presence of EtOH from 0.233 MPa to 0.133 mPa; this is a decrease of approx. 40% and thus near the limit of
 10 insignificance (see Table 6 for comparison). The reason is the limited assay resolution, which for p^* in the studied situation is 20-30%.

Speaking in absolute terms, and making comparison with the magnitude of positive effect on aggregate adaptability caused by Tween 80 (cf. Tables 6
 15 and 7), ethanol in the range disclosed in USP 6 165 500, EP 0475 160 A1 and WO 98/17255 only increases the adaptability of tested aggregates moderately. Similar conclusion is reached by comparing examples 50-61 in USP 6 165 500 (or in the equivalent EP 0475 160 A1).

20 Comparison of the results from experiments 161 and 162 and 157, moreover, confirms that the tested system preservatives (Na metabisulphite; EDTA; BHT, benzyl alcohol) neither affect negatively the desirable extended surface aggregate adaptability nor do they change much the pressure required for driving adequate suspension transport through a nano-porous barrier.

Examples 163-165:Composition:

	75 mg	Phosphatidylcholine from soy-bean (SPC),
5	25 mg	Ketoprofen, sodium (KT)
	See the following table	Tween 80 (mol-% referring to SPC)
	ad 1 ml	Water or 50 mM buffer (pH = 7.2)

Objective: to test the influence of ionic strength of the bulk inorganic electrolyte on the adaptability of mixed amphipat aggregates suspended in such an electrolyte.

Suspension preparation and characterisation. The test suspension was prepared essentially as with examples 147-150, except in that the buffer was sometimes exchanged for water with practically the same pH-value. This had important consequences. When the ionic strength (I) of the bulk electrolyte solution with a pH near 7 changes, ketoprofen distribution and degree of ionisation in Transfersome[®] suspension also changes. This modifies - most probably decreases - extended surface aggregate adaptability, which must be considered when designing products on the basis of given formulation composition. Experimental evidence for this is given in Table 8.

Table 8: The fit results based on formula (*) for the transbarrier flux/driving pressure ratio (barrier penetrability), of various quaternary suspensions with KT as the drug in different buffer systems.

Formulation	p^* [MPa]	P_{max} [10^{-11} m Pa $^{-1}$ ·sec $^{-1}$]	Adaptability a_a , [MPa $^{-1}$]
10 mol-% Tween no buffer	0.49 ± 0.02	212 ± 8	2.041
10 mol-% Tween, 50 mM buffer, $I = 117$ mM	0.25 ± 0.03	230 ± 17	4.000
7.5 mol-% Tween, 6.3% v/v EtOH no buffer	0.31 ± 0.06	194 ± 23	3.226
7.5 mol-% Tween, 6.3% v/v EtOH 50 mM buffer, $I = 117$ mM	0.13 ± 0.01	248 ± 11	7.692
Reference Tween Tfs in the buffer	0.20 ± 0.01	227 ± 3	5.000

Examples 166-167:

5

Composition:

- 75.0 mg Phosphatidylcholine from soy-bean (SPC),
25 mg Ketoprofen, sodium (KT)
12.4 mg Tween 80
10 ad 1 ml Buffer pH = 7.2 and pH = 7.7

Suspension preparation and characterisation: see previous test series.

- Objective: to test the effect of ketoprofen ionisation, which above the pKa(KT)
15 ~ 6.4 increases with pH, on adaptability of the drug loaded mixed lipid
vesicles.

Results: Adaptability of simple formulations containing three amphipatic
components was confirmed to depend on the ionisation state of its only

titratable component, ketoprofen. Detailed results are given in the following Table 9.

Table 9: Fit results, based on eq. (*), for the pressure normalised transbarrier flux of KT-Tfs suspensions at different pH

pH	p^* [MPa]	P_{max} [$10^{-11} \text{ m Pa}^{-1} \cdot \text{sec}^{-1}$]	Adaptability a_a , [MPa $^{-1}$]
7.2	1.66 ± 0.07	345 ± 37	0.602
7.7	0.62 ± 0.07	237 ± 28	1.613
Reference Tfs	0.20 ± 0.01	227 ± 2.9	5.000

Examples 168-169:

10 Composition:

100 mg/ml Phosphatidylcholine from soy-bean (SPC)
as large unilamellar vesicle suspension

254 mg/ml Ketoprofen, sodium (KT) in solution
Buffer pH = 7.2 and pH = 7.7

15 *Mixed during experiments to yield increasing relative ratio of KT in SPC aggregates suspension.*

Objective: to test the ability of ketoprofen to solubilise lipid bilayer membranes.

20

Results: The ability of ketoprofen to solubilise soybean phosphatidylcholine (SPC) membranes was determined by measuring the turbidity of a suspension (10 w-%) of large unilamellar vesicles during successive addition of 1 M solution of ketoprofen. In the first test series this was done in 50 mM

- 117 -

phosphate buffer at $pH = 7.4$, where more than 50% of the drug is ionised and more than 50% of the drug is vesicle-bound, but chiefly in the non-charged form, which does not destabilise lipid membranes significantly. SPC vesicles under tested these conditions were not measurably solubilised, despite the presence of some ionised ketoprofen, but were partly destabilised, as demonstrated in previous examples.

The second experiment was performed at $pH = 11.6$, under which condition all ketoprofen molecules are deprotonated and hence have a maximum solubilisation, i.e. membrane destabilisation, capability. Solubilisation of SPC membranes was now observed when the molar ratio for the drug in vesicle bilayers was above ketoprofen/SPC $\sim 10.8/1$ mole/mole. SPC-ketoprofen association thus produces weakly bound complexes with membrane solubilising capability.

15

Examples 170-174:Composition:

20	75.0 mg	Phosphatidylcholine from soy-bean (SPC, used as a saturated ethanolic solution) the actual number is: 75 mg - Brij content
	25.0 mg	Ketoprofen, sodium (KT)
	See the following table	Brij 98
25	ad 1 ml	Phosphate buffer ($pH = 7.2$)

Objective: to demonstrate the usefulness of another surfactant, Brij, different from Tween 80, to increase the flux through narrow pores of ketoprofen/SPC extended surface aggregates in a suspension.

30

Suspension preparation was essentially the same as in examples 147-150.

- Flux determination. The flux of suspension of extended surface aggregates containing SPC, KT and, in case, Brij 98 was measured using the same device as is used for aggregate adaptability determination. The only difference was that only a single driving pressure was used for suspension characterisation. For comparison, the ratio of KT-loaded and of empty Brij Transfersomes® was calculated (= Rel. Flux).
- 10 The results of the test series measured with Brij 98, a polyoxyethylene-oleyl-ether with 20 OE units in polar head are given in Table 10.

Table 10: Flux of mixed amphipat suspensions through 20 nm pores in a semi-permeable barrier driven by trans-barrier pressure of 0.1 MPa.

Brij 98 content [mol% of SPC]	Flux [mg cm ⁻² sec ⁻¹]	Rel. Flux
0	<1	
2.5	10	>10
5.0	30	>30
7.5	29	>29

15

Examples 175-178:

Composition KT Form(ulation) B (Expt 175):

- 20 Weight-%
- | | |
|-------|---------------------|
| 2.857 | Ketoprofen |
| 7.143 | Phosphatidylcholine |
| 3.000 | Glycerol |

- 119 -

	2.087	Sodium Hydroxide, 50% (FCC)
	0.120	Phosphate buffer salts
	0.100	Linalool
	0.100	Disodium edetate EDTA
5	1.250	Carbomer 974
	0.100	Carbomer 1342
	1.000	Propylen Glycol
	0.200	Ethylparaben
	0.525	Benzyl Alcohol
10	0.020	Butylated hydroxytoluene
	81.499	Water

Composition KT Form.(ulation) A (Expt 176):

Weight-%

15	2.290	Ketoprofen
	6.870	Soy Phosphatidylcholine (SPC)
	0.850	Polysorbate (Tween 80)
	3.651	Ethanol 96%
	0.930	NaOH (sodium hydroxide)
20	0.235	Phosphate buffer salts
	0.050	Sodium metabisulphite
	0.020	Butylhydroxytoluene (BHT)
	0.100	Disodium edetate (EDTA)
	0.250	Methyl parahydroxybenzoate
25	0.525	Benzyl alcohol
	0.100	Linalool
	1.250	Carbomer (Carbopol 980)
	3.00	Glycerol
	79.879	Water

Commercial topical formulation Gabrilen (Expt. 177): according to desk physicians' reference, the preparation contains 25 mg KT/g gel, supplemented with 96% ethanol, 3-propanol, 10% ammonia solution and Carbomer in purified water.

5

Commercial oral formulation Ketoprofen Ratiopharm (KT Ratiopharm) (Expt. 178): according to desk physicians' reference each film tablet contains 50 mg KT in addition to microcrystalline cellulose, gelatine, SiO₂, corn starch, talcum, crosscarmellose sodium, Mg stearate, hypromellose, macrogol, glycerol, dyes E 171 and E 172.

10

Preparation of formulations A and B, which both contained extended surface vesicles, was done essentially as described for examples 147-150.

Commercial comparators were purchased in a local pharmacy and used as obtained.

15

Methodology: The test pigs were numbered and central vein catheters were implanted into the animals. The application area on a hind limb of each animal was shaved with an electric clipper and cleaned with warm water and soap. Then, an application area of 10 cm x 10 cm (= 100 cm²) was marked.

20

At time zero of the sampling period, 2 ml of the blood were sampled from each test animal into a citrate-coated vial to generate plasma. The pigs were anaesthetised for approximately 60 min and the appropriate dose of the test medication was applied onto the application site of a pig or else was given to the animal orally. Further plasma samples (0.5 ml each) were taken 0.5, 1, 2, 3, 5, 8 and 12 hours post application. They were kept frozen until analysis.

25

Ketoprofen concentration was determined with HPLC using standard methods, in case of muscle tissue samples after the specimen

30

homogenisation. Area under the curve (AUC) was calculated by integrating all time-point data.

Results of experiments are given in Tables 11 and Figure 11. Whereas
 5 the individual pharmacokinetic data sets are rather scatterly, yielding
 standard deviations comparable to the mean because of small group
 size, the overall data analysis does demonstrate the superiority of at
 least three amphipat component preparations, in comparison with two
 amphipat component formulations, to deliver an NSAID (ketoprofen)
 10 deep under the application site on the skin. The greater is the
 investigated tissue depth the greater is the observed advantage
 (superficial muscle = 0-1.5 cm; deep muscle > 1.5 cm).

Table 11a: Area under the curve (AUC_{0-8h} [ng x mg⁻¹ x h]), measured with
 15 different KT formulations in pigs

	Gabrilin® (n=4)	Formulaion B (n=7)	Formulation A (n=7)	KT Ratiopharm® (oral, n=3)
Superficial muscle tissue	102	209	306	7
Deep muscle tissue	53	147	301	9

Table 11b: Ketoprofen (KT) concentration in superficial muscle tissue (ng/mg)

Time (hours)	Gabrilien® (n=4)	KT-Tfs Form. B (n=7)	KT-Tfs Form. A (n=7)	KT-Ratiopharm (oral) (n=3)
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
1	5.0 ± 3.3	50.4 ± 48.6	55.5 ± 66.3	1.0 ± 1.2
2	12.8 ± 22.6	75.2 ± 83.8	36.3 ± 32.1	1.6 ± 1.2
3	10.9 ± 11.5	3.0 ± 3.2	25.7 ± 28.5	1.4 ± 0.3
5	19.3 ± 18.7	12.9 ± 11.1	45.2 ± 72.9	0.7 ± 0.2
8	3.8 ± 3.8	19.6 ± 17.9	22.0 ± 17.9	0.2 ± 0.1

5 **Table 11c:** Ketoprofen (KT) concentration in deep muscle tissue (ng/mg)

Time (hours)	Gabrilien® (n=4)	KT-Tfs Form. B (n=7)	KT-Tfs Form. A (n=7)	KT-Ratiopharm (oral) (n=3)
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
1	2.6 ± 2.3	53.4 ± 66.5	24.8 ± 19.0	1.5 ± 1.6
2	5.4 ± 9.3	63.0 ± 51.9	18.8 ± 21.5	1.8 ± 1.0
3	9.0 ± 9.3	1.4 ± 0.8	49.8 ± 71.8	1.6 ± 0.5
5	7.9 ± 5.8	5.6 ± 2.2	49.9 ± 65.0	1.0 ± 0.2
8	2.9 ± 2.9	14.1 ± 10.9	30.2 ± 28.7	0.3 ± 0.2

Examples 179-180:

10 Composition for ketoprofen in carrier suspension (KT-Tfs sol):

Weight-%

3.435 Ketoprofen (KT)

10.305 Soy Phosphatidylcholine (SPC)

- 123 -

	1.275	Polysorbate (Tween 80)
	5.477	Ethanol 96%
	0.533	NaOH (sodium hydroxide)
	0.235	Phosphate buffer salts
5	0.050	Sodium metabisulphite
	0.020	Butylhydroxytoluene (BHT)
	0.100	Disodium edetate (EDTA)
	0.250	Methyl parahydroxybenzoate
	0.525	Benzyl alcohol
10	0.100	Linalool
	3.00	Glycerol
	74.695	Water

Composition for ketoprofen in carrier gel (KT-Tfs gel):

15

As in experiment 179, except in that the first four components are diluted 1.5-fold and Carbomer (Carbopol 980), buffered to pH = 7.2, is included to final concentration of 1.25 w-%.

20

Objective: to test the effect of formulation viscosity, and the presence of a thickening agent as viscosity modifier, on the ability of NSAID loaded extended surface aggregates to deliver the drug (ketoprofen) deep under the application site on the skin.

25

Methodology was the same as in experiments 175-178, except in that no oral comparator was included. A total of 4 pigs were used in each group.

30

Area under the curve (AUC) was calculated by integrating all PK (pharmacokinetic) data measured in different tissues (plasma, not shown) and the muscles under drug application site on the skin. The

results obtained for superficial (0-1.5 cm) and deep (> 1.5 cm) muscle are given in Tables 12, and suggest no detrimental effect of the thickening agents used in KT-Tfs gel to achieve the desired suspension viscosity of approx. 730 mPa s. If anything, the thickening agent present in the tested gel is beneficial.

Table 12a: Area under the curve (AUC_{0-8h} [$ng\ mg^{-1}\ h$]), measured with two carrier-based ketoprofen (KT) formulations in pigs

	KT-Tfs gel 17 mg (n=4)	KT-Tfs sol. 17 mg (n=4)	KT-Tfs gel 50 mg (n=4)	KT-Tfs sol. 50 mg (n=4)
Superficial muscle tissue	147	44	278	186
Deep muscle tissue	97	63	266	202

Table 12b: KT concentration in superficial muscle tissue (ng/mg)

Time (hours)	KT-Tfs gel 17 mg (n=4)	KT-Tfs sol. 17 mg (n=4)	KT-Tfs gel 50 mg (n=4)	KT-Tfs sol. 50 mg (n=4)
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
1	83.3 ± 82.9	2.3 ± 1.5	55.5 ± 66.3	23.0 ± 29.3
2	24.1 ± 27.5	0.8 ± 0.3	36.3 ± 32.1	21.2 ± 33.6
3	8.1 ± 8.0	2.8 ± 0.1	25.7 ± 28.5	9.0 ± 2.1
5	14.2 ± 14.2	10.6 ± 12.5	45.2 ± 72.9	34.8 ± 49.8
8	3.1 ± 2.6	3.5 ± 2.4	22.0 ± 17.9	29.8 ± 50.1

Table 12c: KT concentration in deep muscle tissue (ng/mg)

Time (hours)	KT-Tfs gel 17 mg (n=4)	KT-Tfs sol. 17 mg (n=4)	KT-Tfs gel 50 mg (n=4)	KT-Tfs sol. 50 mg (n=4)
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
1	36.0 ± 49.1	14.7 ± 1.5	24.8 ± 19.0	24.5 ± 44.7
2	19.4 ± 23.5	0.8 ± 0.3	18.8 ± 21.5	4.5 ± 4.0
3	2.4 ± 2.6	9.2 ± 3.1	49.8 ± 71.8	25.4 ± 43.0
5	13.5 ± 8.8	9.3 ± 12.5	49.9 ± 65.0	46.6 ± 85.6
8	2.4 ± 1.4	6.4 ± 2.4	30.2 ± 28.7	15.6 ± 23.4

Claims

- 5 1. Preparation based on a combination of at least one first
(membrane forming component MFC), at least one second (membrane
destabilising component MDC), and at least one third (membrane
destabilising component MDC) amphipatic component suspended in a
suitable liquid medium in the form of corresponding mixed amphipat
10 aggregates with extended surface (ESAs) with one or a few mixed amphipat
coating(s), which are preferably bilayer like, wherein said ESAs formed by a
combination of
- all three said components have surfaces in contact with said liquid
medium that are at least 50% more extended, on the average, than the
15 typical surfaces of aggregates comprising the said at least one second
and at least one third amphipatic component alone, at the same
concentrations and, in case, after adjustment for the physico-chemical
effects of resulting from the absence of said first amphipatic compound
(MFC)
- 20 for application, administration or transport of an active ingredient, which can
be one of the three amphipatic components, especially for biological,
medical, immunological, or cosmetic purposes, into and through the pores in
semi-permeable barriers or other constrictions, such as through the skin of
warm blood creatures or the like.
- 25 2. A combination of at least one first (membrane forming component
MFC), at least one second (membrane destabilising component MDC), and
at least one third (membrane destabilising component MDC) amphipatic
component suspended in a suitable liquid medium in the form of mixed
30 amphipat aggregates with extended surface (ESAs) with one or a few mixed
amphipat coating(s), which are preferably bilayer like, wherein the

- 127 -

- said at least one first substance has a tendency to self aggregate and is at least 10-times less soluble in said liquid medium than said at least one second and said one third substance, allowing the first to form extended surfaces,
 - 5 - said at least one second substance is at least 10-times more soluble than said at least one first substance in said liquid medium and, on its own, tends to form or supports the formation of surfaces that are at least 2-times less extended than the surfaces containing the at least one first substance alone,
 - 10 - said at least one third substance being also at least 10-times more soluble in said liquid medium than the first substance and optionally forms self-aggregates with aggregation number at least 10-times smaller than that of self-aggregates of said first substance; and
 - said extended surfaces comprising said at least one first, at least one
15 second and at last one third substance, in equilibrium, have at least 50% greater surface than the surfaces formed by the at least one second or one third substance alone, at the same concentration and, in case, after adjustment for the physico-chemical effects of the absence of said first amphipatic compound (MFC)
 - 20 for a preparation for application, administration or transport of at least one active ingredient , which can be one of the three amphipatic components, especially for medicinal or biological purposes, into and through barriers and constrictions, such as the skin of warm blood creatures or the like.
- 25 3. Extended-surface aggregates (ESAs) comprising at least one first (membrane forming component, MFC), at least one second (membrane destabilising component, MDC), and at least one third (membrane destabilising component, MDC), all of which are amphipatic, suspended in a suitable liquid medium, which permits said ESAs to permeate barriers with
- 30 the pores with at least 40% smaller radius than the average ESAs radius, as

measured after the ESAs have permeated the barrier pores and assuming spherical ESAs geometry.

4. Preparation based on a combination of at least one first
5 (membrane forming component MFC), at least one second (membrane destabilising component MDC), and at least one third (membrane destabilising component MDC) amphipatic component suspended in a suitable liquid medium in the form of corresponding mixed amphipat aggregates with an extended surface (ESAs) with one or a few, preferably
10 bilayer-like, mixed amphipat coating(s), wherein said MFC alone forms extended-surface aggregates with aggregation number of at least 5000, and preferably more than 10.000, and both MDCs alone and the combination of both MDCs form smaller aggregates with no substantially extended surface and aggregation number below 5000, and preferably below 1000 in contact
15 with said suitable liquid medium.

5. A combination according to claims 1 to 4,
wherein the said extended surfaces are in the form of membrane surfaces.

- 20 6. A combination according to any of preceding claims,
wherein the said at least one second substance increases the flexibility of extended surfaces comprising said at least one first, at least one second, and at least one third substance in comparison with the surfaces formed merely by an at least one first substance or else with the surfaces formed by at least
25 one first and at least one third substance.

7. A combination according any of preceding claims,
wherein the said at least one second and one third substance together increase the permeability of extended surfaces containing the said at least
30 one first, at least one second, and at least one third substance, in

comparison with the surfaces formed merely by the at least one first substance or else with the surfaces formed by at least one first and at least one third substance.

5 8. A combination according to any of preceding claims,
wherein the said at least one second substance or the said at least one third
substance increases the ability to tolerate high curvature, as assessed by
relative stability of said extended surface comprising said one first, said one
second and said one third substance against enforced higher curvature
10 during passing through a constriction with maximum diameter at least 1.4
times smaller than the average diameter of an extended surface formed by
an at least one first substance alone.

 9. A combination according to any of preceding claims,
15 wherein the at least one first substance and the at least one second
substance or the at least one third substance differ in solubility on the
average at least 10-fold.

 10. A combination according to any of preceding claims,
20 wherein the at least one second substance and the at least one third
substance differ in solubility on the average at least 2-fold.

 11. A combination according to any of preceding claims,
wherein the at least one second substance or the at least one third
25 substance have the hydrophilicity-lipophilicity ratio between 10 and 20.

 12. A combination according to any of preceding claims,
wherein the concentration of said at least one second substance used in the
combination with said one first and said one third substance is below 80% of
30 the concentration that would be needed to render the aggregates comprising

only said one first and said one second substance as adaptable to ambient stress as the selected combination of all at least three substances, whereby the said one second and said one third substance can exchange roles.

5 13. A combination according to any of preceding claims, wherein the concentration of said at least one second substance or of said at least one third substance, as the case may be, amounts to at least 0.1% of the relative concentration as defined in claim 8.

10 14. A combination according to any of preceding claims, wherein the concentration of said at least one second or of said at least one third substance amounts to 1 – 80% of the relative concentration as defined in claim 8.

15 15. The combination according to any of preceding claim, wherein relative concentration of said at least one third substance used in combination with said one first and said one second substance is above 0.1% of maximum possible concentration of the said at least one third substance in the system,

20 a) as defined in terms of the solubility of said third substance in the system or in said at least three-component aggregates, or else
b) as determined by the negative action of said at least one third substance on the stability of said at least three-component aggregates,

25 whereby the said one third and one second substance can also exchange roles.

 16. The combination according to any of preceding claims, wherein relative concentration of said at least one third substance used in
30 combination with said one first and with said one second substance is

between 1% and 99% of maximum possible concentration of said at least one third substance,

a) as defined in terms of the solubility of said third substance in the system or in said at least three-component aggregates,

5 b) or else as determined by the detrimental effect of said at least one third substance on the stability of said at least three-component aggregates,

whereby the said one third and one second substance can also exchange roles.

10

17. The combination according to any of preceding claims, wherein relative concentration of said at least one third substance used in combination with the said one first and the said one second substance is between 10% and 95% of the maximum possible concentration of said at least one third substance, as defined in terms of the third substance solubility in the system or in said aggregates, or else as determined by the detrimental effect of said at least one third substance on the stability of said at least three-component aggregates, whereby the said one third and one second substance can also exchange roles.

20

18. The combination according to any of preceding claims, wherein relative concentration of said at least one third substance used in combination with the said one first and the said one second substance is between 25% and 90% of the maximum possible concentration of said at least one third substance, as defined in terms of the third substance solubility in the system or in said aggregates, or else as determined by the detrimental effect of said at least one third substance on the stability of said at least three-component aggregates, whereby the said one third and one second substance can also exchange roles.

30

19. The combination according to any of preceding claims, wherein the total dry mass of all at least three amphipatic substances, which together form highly adaptable aggregates with an extended surface, is between 0.01 weight-% and 50 weight-%.

5

20. The combination according to any of preceding claims, wherein total dry mass of all at least three substances, which together form highly adaptable aggregates with an extended surface, is between 0.5 weight-% and 30 weight-%.

10

21. The combination according to any of preceding claims, wherein total dry mass of all at least three substances, which together form highly adaptable aggregates with an extended surface, is between 1 weight-% and 15 weight-%.

15

22. A combination according to any of preceding claims, wherein the extended surfaces with a high adaptability, which contain said at least three substances, have an average curvature corresponding to an average radius between 15 nm and 5000 nm.

20

23. A combination according to any of preceding claims, wherein the extended surfaces with a high adaptability, which contain said at least three substances, have an average curvature corresponding to an average radius between 30 nm and 1000 nm.

25

24. A combination according to any of preceding claims, wherein the extended surfaces with a high adaptability, which contain said at least three substances, have an average curvature corresponding to an average radius between 40 nm and 300 nm.

30

25. A combination according to any of preceding claims, wherein the extended surfaces with a high adaptability, which contain said at least three substances, have an average curvature corresponding to an average radius between 50 nm and 150 nm.

5

26. The combination of substances according to any of preceding claims, wherein the concentration and the composition of the electrolyte in which the extended surfaces with at least one first, at least one second, and at least one third substance are suspended, and which comprises mono and/or oligovalent ions, is chosen to have ionic strength between $I = 0.001$ and $I = 1$.

10

27. The combination of substances according to any of preceding claims, wherein the concentration and the composition of the electrolyte, in which the extended surfaces with at least one first, at least one second, and at least one third substance are suspended, and which comprise mono and/or oligovalent ions, is chosen to have pH value

15

20

a) in the vicinity of the logarithm of the apparent ionisation constant (pK_a) of said at least one second substance, if the latter is mono-ionizable, or in the vicinity of such pK_a value that maximises the solubility of said at least one second substance, if the latter has several ionizable groups, or else

25

b) in the vicinity of pH optimum for the most rapidly decaying or the otherwise most sensitive amongst the said at least three substances, if the said at least one second substance is not ionizable.

30

28. The combination of substances according to any of preceding claims,

wherein the pH value of the polar medium in which the ESAs comprising at least one first, at least one second, and at least one third substance are suspended is between $pH = pKa - 3$ and $pH = pKa + 3$.

5 29. The combination according to any of preceding claims, wherein the at least one first substance being less soluble in the liquid medium, and/or being the surface-building substance in the system, is a lipid, whereas the at least one second substance being more soluble in the liquid medium and/or increasing the tolerable surface curvature or adaptability of
10 said extended surface, is a membrane destabilising amphipat, which is typically a surfactant, and said at least one third substance is either a biologically active amphipatic ingredient, which has a capability of its own to increase the tolerable surface curvature, or adaptability of said extended surface, or else is a different surfactant different from the said at least second
15 substance.

30. The combination according to any of preceding claims, wherein the molecules are arranged in the form of minute fluid droplets suspended or dispersed in a liquid medium and surrounded by a coating of
20 one or several layers of the at least one first substance, which is capable of self-aggregation, and of at least one second substance and of at least one third substance, which are both amphipatic, such that

a) the former substance and the latter two substances differ in solubility in a suitable liquid medium at least 10-fold, or such that

25 b) the average radius of homo-aggregates of the more soluble amongst the at least one second and third substance or of hetero-aggregates of the at least one first, the at least one second and the at least one third substance is smaller than the average radius of homo-aggregates of said at least one first substance, which is the least soluble amongst the three.

31. Combination according to any of preceding claims,
wherein the at least one first substance is a polar or a non-polar, surface -
forming lipid.

5

32. Combination according to any of preceding claims, wherein the at
least one first substance in extended surfaces is capable of forming bilayer
membranes and preferably forms bilayers on its own.

10

33. Combination according to any of preceding claims,
wherein the solubility of the at least one first substance in a polar liquid
medium is between 10^{-12} M and 10^{-7} M.

15

34. Combination according to any of preceding claims,
wherein the at least one first substance forming extended surfaces is
selected from the group comprising lipids, lipoids from a biological source,
corresponding synthetic lipids, or modifications thereof.

20

35. Combination according to any of preceding claims,
wherein said at least one first substance forming extended surfaces is
selected from the group comprising glycerides, glycolipids,
glycerophospholipids, isoprenoidlipids, sphingolipids, steroids, sterines or
sterols, sulphur-containing lipids, lipids containing at least one carbohydrate
residue, or other polar fatty derivatives.

25

36. Combination according to any of preceding claims,
wherein said at least one first substance forming extended surfaces is
selected from the group comprising phosphatidylcholines,
phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols,
30 phosphatidic acids, phosphatidylserines, sphingomyelins,

sphingophospholipids, glycosphingolipids, cerebroside, ceramidpolyhexosides, sulphatides, sphingoplasmalogenes, or gangliosides.

37. Combination according to any of preceding claims,

5 wherein said extended surface-forming substance is selected from the group comprising lipids with one or two, not necessarily identical, fatty chains, especially with acyl-, alkanoyl-, alkyl-, alkylene-, alkenoyl-, alkoxy, or chains with omega-cyclohexyl-, cyclo-propane-, iso- or anteiso-branched segments, or the corresponding chains mixtures.

10

38. Combination according to any of preceding claims,

wherein said substance that forms extended surfaces is selected from the group comprising lipids with *n*-decyl, *n*-dodecyl (lauryl), *n*-tetradecyl (myristyl), *n*-hexadecyl (cetyl), *n*-octadecyl (stearyl), *n*-eicosyl (arachinyl), *n*-docosyl (behenyl) or *n*-tetracosyl (lignoceryl), 9-*cis*-dodecenyl (lauroleyl), 9-*cis*-tetradecenyl (myristoleyl), 9-*cis*-hexadecenyl (palmitoleinyl), 9-*cis*-octadecenyl (petroselinyl), 6-*trans*-octadecenyl (petroselaidinyl), 9-*cis*-octadecenyl (oleyl), 9-*trans*-octadecenyl (elaidinyl), 9-*cis*-eicosenyl (gadoleinyl), 9-*cis*-docosenyl (cetoleinyl) or 9-*cis*-tetracosoyl (nervonyl), *n*-decyloxy, *n*-dodecyloxy (lauryloxy), *n*-tetradecyloxy (myristyloxy), *n*-hexadecyloxy (cetyloxy), *n*-octadecyloxy (stearyloxy), *n*-eicosyloxy (arachinyloxy), *n*-docosoyloxy (behenyloxy) or *n*-tetracosoyloxy (lignoceryloxy), 9-*cis*-dodecenyloxy (laurolexyloxy), 9-*cis*-tetradecenyloxy (myristolexyloxy), 9-*cis*-hexadecenyloxy (palmitoleinyloxy), 6-*cis*-octadecenyloxy (petroselinyloxy), 6-*trans*-octadecenyloxy (petroselaidinyloxy), 9-*cis*-octadecenyloxy (olexyloxy), 9-*trans*-octadecenyloxy (elaidinyloxy), and 9-*cis*-eicosenyl (gadoleinyloxy), 9-*cis*-docosenyl (cetoleinyloxy) or 9-*cis*-tetracosoyl (nervonyloxy), *n*-decanoyloxy, *n*-dodecanoyloxy (lauroyloxy), *n*-tetradecanoyloxy (myristoyloxy), *n*-hexadecanoyloxy (palmitoyloxy) *n*-octadecanoyloxy (stearoyloxy), *n*-

15

20

25

30

eicosanoyloxy (arachinoyloxy), *n-n*-docosoanyloxy (behenoyloxy) and *n*-tetracosanoyloxy (lignoceroyloxy), 9-*cis*-dodecenoyloxy (lauroleoyloxy), 9-*cis*-tetradecenoyloxy (myristoleoyloxy), 9-*cis*-hexadecenoyloxy (palmitoleinoyloxy), 6-*cis*-octadecenoyloxy (petroselinoyloxy), 6-*trans*-octadecenoyloxy (petroselaidinoyloxy), 9-*cis*-octadecenoyloxy (oleoyloxy), 9-*trans*-octadecenoyloxy (elaidinoyloxy), and 9-*cis*-eicosenoyloxy (gadoleinoyloxy), 9-*cis*-docosenoyloxy (cetoleinoyloxy) and 9-*cis*-tetracosenoyloxy (nervonoyloxy) or the corresponding sphingosine derivative chains.

10

39. Combination according to any of preceding claims, wherein said at least one second substance is a surfactant.

15

40. Combination according to any of preceding claims, wherein said surfactant is selected from the group comprising nonionic, zwitterionic, anionic and cationic surfactants.

20

41. Combination according to any of preceding claims, wherein said surfactant has the solubility in a polar liquid in which the extended surfaces are prepared between 10^{-6} M and 10^{-2} M.

25

42. Combination according to any of preceding claims, wherein said surfactant is selected from the group comprising long-chain fatty acids or long chain fatty alcohols, long chain fatty ammonium salts, such as alkyl- or alkenoyl-trimethyl-, -dimethyl- and -methyl-ammonium salts, alkyl- or alkenoyl-sulphate salts, or monovalent salts of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, acyl- or alkenoyl-dimethyl-aminoxides, long fatty chain, for example alkanoyl, dimethyl-aminoxides and especially dodecyl dimethyl-aminoxide, long fatty chain, for example alkyl-N-, methylglucamides and alkanoyl-N-

30

methylglucamides, long fatty chain-N,N-dimethylglycines, for example N-alkyl-N,N-dimethylglycines, 3-(long fatty chain-dimethylammonio)-alkanesulphonates, for example 3-(acyldimethylammonio)-alkanesulphonates, long fatty chain derivatives of sulphosuccinate salts, long fatty chain-sulphobetaines, for example N-acyl-sulphobetaines, long fatty chain betaines, polyethylen-glycol-acylphenyl ethers, polyethylene-long fatty chain-ethers such as polyethylene-acyl ethers, polyethyleneglycol-iso long fatty chain ethers, such as polyethyleneglycol-isoacyl ethers, polyethyleneglycol-sorbitane-long fatty chain esters, for example polyethyleneglycol-sorbitane-acyl esters and especially polyethyleneglycol-monolaurate (e.g. Tween 20), polyethyleneglycol-sorbitan-monooleate (e.g. Tween 80), polyhydroxyethylene-long fatty chain ethers, for example polyhydroxyethylene-acyl ethers (Brij series), or the corresponding polyhydroxyethylene-acyl esters (Myrj series) and polyethoxylated castor oil 40 (Cremophor EL), sorbitane-mono long fatty chain, for example alkylate (Arlacel or Span series), long fatty chain -N-methylglucamides, such as acyl-N-methylglucamides or alkanoyl-N-methylglucamides, long fatty chain sulphates, for example alkyl-sulphates and their salts; long fatty chain thioglucosides, such as alkylthioglucosides, long fatty chain derivatives of various carbohydrates, such as pentoses, hexoses and disaccharides, especially alkyl-glucosides and maltosides; further lysolipids, such as long fatty chains derivatives of common phospholipids, especially lyso-glycerophosphatidylcholine (= lysolecithin), lyso-glycerophosphatidylethanolamine (lysokephalin), lyso-glycerophosphatidic acid, lyso-glycerophosphorylglycerol, lyso-glycerophosphorylserine, corresponding short-chain phospholipids, and membrane destabilising oligo or polypeptides.

43. Combination according to any of preceding claims,
wherein the at least one second substance is charged if the at least one third
substance is uncharged, and the at least one second substance is uncharged
if the at least one third substance is charged, similar preferred combinations
5 also being possible for the said at least one first and one second or for the
said at least one first and one third substance.

44. Combination according to any of preceding claims,
wherein the surface, formed by the at least one first, one second and one
10 third substance, at least one of which is charged, contains between 1% and
75% of the charged component.

45. Combination according to any of preceding claims,
wherein the surface, formed by the at least one first, one second and one
15 third substance, at least one of which is charged, contains between 5% and
50% of the charged component.

46. Combination according to any of preceding claims,
wherein the surface, formed by the at least one first, one second and one
20 third substance, at least one of which is charged, contains between 10% and
30% of the charged component.

47. Combination according to any of previous claims,
wherein the surface-supporting at least one first substance is a
25 phosphatidylcholine, a phosphatidylethanolamine-N-mono- or N-di-methyl,
phosphatidic acid or its methyl ester, phosphatidylserine and/or
phosphatidylglycerol and the at least one second substance which on its own
forms small aggregates is a lysophospholipid, especially a lysophosphatidic
acid, lysomethylphosphatidic acid, lysophosphatidylglycerol,
30 lysophosphatidylcholine, a partially N-methylated

lysophosphatidylethanolamine, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurocholate, or a sufficiently polar sterol derivative, a laurate, myristate, palmitate, oleate, palmitoleate, elaidate or other long-chain fatty acid salt and/or a Tween-, a Myrj-, or a Brij-surfactant, or a Triton, a long-chain fatty sulphonate, -sulphobetaine, -N-glucamide or -sorbitane (Arlacel or Span) surfactant.

48. Combination according to any of preceding claims, wherein the at least one third substance, if not a surfactant different from the at least one second substance, but otherwise selected from similar surfactant classes, is a biologically active amphipat which can destabilise lipid membranes.

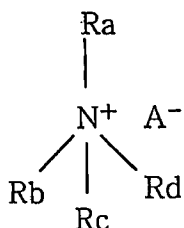
49. Combination according to any of preceding claims, wherein the solubility of at least one third or of one second substance in a polar liquid is between 5×10^{-6} M and 1 M.

50. Combination according to any of preceding claims, wherein the at least one third amphipat or the at least one second amphipat adsorbs to the surface of lipid bilayer membrane but is well miscible with or soluble in the polar liquid in which the said extended surfaces are formed.

51. Combination according to any of previous claims, wherein the at least one third or one second substance is a drug.

52. Combination according to any of preceding claims, wherein the amphipatic compound with biological activity, which can act as a drug, is a substituted ammonium compound of the formula

- 141 -



(1)

in which

- 5 a) Ra represents a hydrophobic group, and Rb, Rc, and Rd, independently of one another, each represents hydrogen, C1-C4-alkyl, 2-hydroxyethyl, allyl or cyclo-C3-C6-alkyl-C1-C3-alkyl, or two of the radicals Rb, Rc and Rd together represent C4- or C5- alkylene interrupted by -HN-, -N(C1-C4-alkyl)-, -N(2-hydroxyethyl)- or by
- 10 oxygen, or;
- b) Ra and Rb are two hydrophobic groups or together represent a hydrophobic group, and Rc and Rd, independently of one another, each represents hydrogen, C1-C4-alkyl, allyl or cyclo-C3-C6-alkyl-C1-C3-alkyl, or
- 15 c) Ra, Rb and Rc together represent a hydrophobic group, and Rd represents hydrogen or C1-C4-alkyl, and A⁻ represents the anion of a pharmaceutically acceptable acid, as a carboxylic acid salt of the formula



in which Ra represents a hydrophobic group, and Y⁺ represents the cation of a pharmaceutically acceptable base, as an alpha-amino acid compound of the formula

25

- 142 -



in which Ra represents a hydrophobic group, and Rb and Rc,
 5 independently of one another, each represents hydrogen or C1-C4-alkyl, as a
 phosphoric acid monoester of the formula



10

in which Ra represents a hydrophobic group and Y⁺ represents the
 cation of a pharmaceutically acceptable base, or as an acid addition
 salt of a compound having a hydrophobic group Ra and an
 15 imidazoline, imidazolidine or hydrazino group as hydrophilic group.

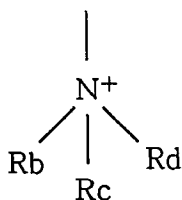
53. Combination according to any of preceding claims,
 wherein the said at least one third or one second amphipatic substance with
 biological activity, which can act as a drug, is a substituted ammonium
 20 compound of the formula 1 in which

- a) the hydrophobic group can be an aliphatic hydrocarbon radical that
 can be interrupted by an oxygen or sulphur atom, may contain the
 groups -CO(=O)-, -O-C(=O)-, -C(=O)-NH-, -O-C(=O)-NH- or hydroxy,

- 143 -

and can be substituted by from 1 to 3 monocyclic, aliphatic or aromatic hydrocarbon radicals, by a bi- or tri-cyclic, aromatic or partially saturated hydrocarbon radical, by a monocyclic, aromatic, partially saturated or saturated heterocycle or by a bi- or tri-cyclic, aromatic, partially saturated or benzo-fused heterocycle, or can be a monocyclic, aliphatic or aromatic hydrocarbon radical or a bicyclic, aliphatic or benzo-fused hydrocarbon radical, and the hydrophilic group is a group of the formula

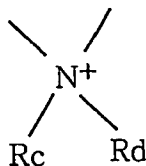
10



in which Rb, Rc, and Rd, independently of one another, each represents hydrogen, C1-C4-hydrogen, C1-C4-alkyl or 2-hydroxyethyl, or in which two of the radicals Rb, Rc and Rd together represent piperidino, piperazinyl, 1-methylpiperazinyl, 1-(2-hydroxyethyl-piperazinyl) or morpholino, and the other radical represents hydrogen, or,

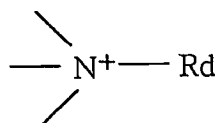
b) the hydrophobic groups Ra and Rb can be two aliphatic hydrocarbon radicals which can be substituted by one or two monocyclic, aliphatic or aromatic hydrocarbon radicals or by substituted, monocyclic, aromatic, partially saturated or saturated heterocycle, or Ra and Rb together represent a monocyclic, aromatic, saturated, partially saturated or benzo-fused heterocycle, and the hydrophilic group is a group of the formula

- 144 -



- in which Rc and Rd, independently of one another each represents
 5 hydrogen or C1-C4-alkyl, or
 c) the hydrophobic group is formed by Ra, Rb and Rc together and
 represents an aromatic, partially saturated or benzo-fused heterocycle and the
 hydrophilic group is a group of the formula

10



- in which Rd represents hydrogen or C1-C4-alkyl, preferably methyl,
 15 and A⁻ is the anion of a pharmaceutically acceptable acid, or a
 carboxylic acid salt of the formula 2 in which the hydrophobic group
 Ra can be an aliphatic hydrocarbon radical, which can be substituted
 by a monocyclic, aromatic hydrocarbon radical, or by a bi- or tri-cyclic,
 aromatic or partially saturated hydrocarbon radical, by a monocyclic,
 20 aromatic or partially saturated heterocycle or by a bi- or tri-cyclic,
 aromatic, partially saturated or benzo-fused heterocycle or by a steroid
 radical, or Ra can be a monocyclic, aromatic hydrocarbon radical, a bi-
 or tri-cyclic, aromatic or partially saturated hydrocarbon radical, a
 monocyclic, aromatic or partially saturated heterocycle or a bi- or tri-
 25 cyclic, aromatic, partially saturated or benzo-fused heterocycle, and Y⁺
 is the cation of a pharmaceutically acceptable base.

54. Combination according to any of preceding claims,
wherein the said at least one third or one second amphipatic substance,
which acts as a drug, is a substituted ammonium compound or the
5 corresponding amino compound that can be converted into the ammonium
compound by salt formation, such as acetylcholine chloride, methacholine
chloride, carbachol, muscarine, pilocarpine, arecoline, physostigmine,
neostigmine, pyridostigmine bromide, serotonin, histamine, tryptamine,
bufotenine, psilocybin, morphine, hydromorphone, oxymorphone,
10 levorphanol, codeine, hydrocodone, oxycodone, nalorphine, naloxone,
naltrexon, buprenorphine, butorphanol, nalbuphine, pholcodine, pentazocine,
ketamine, metazocine, pentazocine, cyclazocine, pethidine, cetobemidon,
alphaphrodine, ethoheptazine, prodilidine, profadol, methadone,
normethadone, isomethadone, dipipanone, phenadoxone, dimephethanol,
15 dextromoramide, D-propoxyphene, 1-benzyl-2-dimethylaminomethyl-1-
propanoyloxytetralin, tramadol, dimethylthiambutene, diampromide,
phenampromide, propiram, tilidine, metopoline, etonitazene, ergotamine,
dihydroergotamine, dihydroergocryptine, methysergide, lisuride, dimetotiazin,
dizotifen, oxetoron, cyproheptadine, procaine, chlorprocaine,
20 hydroxyprocaine, propoxycaine I oxy-buprocaine, propoxymetacaine,
piridocaine, leucinocaine, butacaine p tetracaine, hydroxytetracaine,
cornecaine, edan, piperocaine, cyclomethycaine, parethoxysaine, stadacain,
cinchocaine, lidocaine, pyrrocaine, granocaine, butanillicaine, tolycaine,
mepivacaine, bupivacaine, prilocaine, carticaine, dipiperidon, propicocaine,
25 dyclonine, pramocaine, fomocaine, quinisocaine, profenamine,
promethazine, periciazine, perimethazine, chlorpromazine, perphenazine,
prochlorperazine, triflumpromazine, trifluoperazine, fluphenazine,
thioridazine, mesoridazine, piperacetazine, acetophenazine, ethymemazine,
dimethacrine, opipramol, clomipramine, imipramine, desimipramine,
30 trimipramine, chloroprothixene, thiothixene, amitriptyline, nortriptyline,

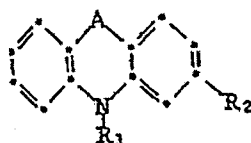
- doxepin, thiepin, protriptyline, prothipendyl, femoxetin, citalopram, zimelidine, trebenzomin, viloxazine, nomifensine, femoxetin, tranlycycgromine, pargyline, etryptamine, flurazepam, mescaline, Nalpha,Nalpha-dimethyl-tryptamine, bufotenine, psilocin, psilocylin, scopolamine, atropine, benztropine,
- 5 trihexyphenidyl, cycrimine, pridinol, biperidine, procyclidine, caramiphen, phenglutarimide, orphenadrine, chlor-phenoxamine, metixen, doxapram, amphetamine, methamphetamine, propylhexedrine, prolintane, fencamfamine, methylphenidol, pipradrol, phenmetrazine, diethylpropion, meclofenoxat, naftidrofuryl, dexamphetamine, phentermin, chlorphentermine,
- 10 fenfluramine, amfepramone, phenmetrazine, phendimetrazine, tubocumarin, alcuronium chloride, gallamin triethiodide, hexacarbacholine bromide, pancuronium bromide, suxamethonium chloride, decamethonium bromide, scopolamine butyl bromide, bevonium methyl sulphate, valethamate bromide, methanteline bromide, camylofine, hexahydroadiphenine, adiphenine,
- 15 fencarbamide, benzyclamine, ditaxol, chloroquine, tamoxifen, ethamoxytriphetol, phenbenzamine, tripelenamin, chlorpyramine, mepyramine, metaphenilene, metapyrilene, chloropyrilene, histpyrroclin, bamipin, thenalidine, clemizole, meth-dilazine, isothipendyl, oxomenazine, diphenhydramine, medrylamine, chlorophenoxamine, silachlorophenoxamin,
- 20 carbinoxamine, diphenpyraline, clemastine, ametho-benzepine, pheniramine, chlorophenamine, bromo-pheniramine, triprolidine, cycliramine, phenindamine, dimetindene, cyproheptadine, ketotifen, epinephrine (adrenaline), norepinephrine (noradrenaline), dopamine, nordefrin, ethylnorepinephrine, isoprenaline, iso--ethorine, metaproterenol,
- 25 orciprenaline, metaraminol, phenylephrine, hydroxyamphetamine, methoxyphenamine, methoxamine, albuterol, ephedrine, norephedrine, fenfluramine, phenylpropanolamine, pholedrine, tyramine, dichloroisoprenaline, norfenefrine, octopamine, etilefrin, acebutolol, atenolol, meto-prolol, toliprolol, alprenolol, oxprenolol, bunitrolol, bupranolol, talinolol,
- 30 phenbutolol, bufetolol, varbian (R,S- or S-form), propanolol, indenolol,

pindolol, mepindolol, nadolol, bunolol, sofalol, nifenalol, cabetalol, bufenalol, reserpine, rescinnamine, syringopine, chlorotetracycline, oxytetracycline, tetracycline, demethylchlorotetracycline, metacycline, doxycycline, minocycline, rolitetracycline, quinine, conquinidine, quinidine, cinchonine,
 5 pamaquine, prlmaquine, pentaquine, chloroquine, santoquine, hydroxychloroquine, amodiaquine, mepacrin, biguanid-1,3,5-triazin, proguanil, bromoguanil, chloroproguanil, nitroguanil, cycloguanilembonate, pyrimethamine, tri-methoprim, lucanthone, hycanthone, miracil A or B, amantadine, cyclooctylamine, rimantadin, prednisolone diethylaminoacetate.

10

55. Combination according to any of preceding claims, wherein the said at least one third or one second amphipatic substance takes the role of a drug as the substituted ammonium compound or as the corresponding amino compound that can be converted into the ammonium
 15 compound by salt formation, and is a compound selected from the group of the acid addition salts of antidepressants of the formula in which R1 represents lower alkyl, for example methyl, A represents the group N- ν R1, oxygen or sulphur, and R2 represents hydrogen or cyano; acid addition salts of antidepressants of the formula

20



(1.10)

in which R1 represents lower alkylamino-lower alkyl, for example 3-methylamino-n-propyl, di-lower alkyl-amino-lower alkyl, for example 3-dimethylamino-n-propyl or 3-(4-(2-hydroxyethyl)-piperazin-1-yl)-n-propyl and A
 25 represents ethylene or vinylene, or acid addition salts of amphetamine,

methamphetamine, benzphetamine, propyl-hexedrine, prolintan, fencamfin, methylphenidate, pipradrol, phenmetrazine, adiphenine, epinephrine, norepinephrine, dopamine, nordefrin, ethyl-norepinephrine, isoprenaline, isoethorine, meta-proterenol, orciprenaline, metaraminol, phenylephrine, 5 hydroxyamphetamine, methoxyphenamine, ephedrine, norephedrine, pholedrine, tyramine, norfenefrin, octopamine, acebutolol, atenolol, toliprolol, alprenolol, oxprenolol, bunitrolol, bupranolol, talinolol, phenbutolol, bufetolol, varbian (R,S-form and S-form), reserpine, rescinnamine, syringopine or prednisolone diethylaminoacetate.

10

56. Combination according to any of preceding claims, wherein the said at least one third or second amphipatic substance takes the role of a drug as the substituted ammonium compound of the formula 1 or as the corresponding amino compound that can be converted into the ammonium compound by salt formation, 1-(2R-2-hydroxy-3- 15 methylaminopropyl)dibenzo[b,e]bicyclo[2.2.2]octadiene, and the 2R,S-isomeric mixture, maprotiline, benzoctamine, 3-methyldibenzo[2,3:6,7]-oxepino[4,5-diazepine hydrochloride, 7-cyano-3-methyl-2,3,4,5- tetrahydro-1H-dibenzo[2,3:6,7]-thiepino[4,5-d]azepine methanesulphonate, 3,10- 20 dimethyl-1,2,3,4,5,10-hexahydrodibenzo[b,f]azepino[4,5]azepine maleate, clomipramine, opipramol, desipramine, imipramine or imipramine N-oxide, ephedrine, norephedrine, 1-iso-propylamino-3-[4-(2-methylthioethoxy)-phenoxy]-propan-2-ol, 1-isopropylamino-3-(2-pyrrol-1-ylphenoxy)-propan-2-ol, oxprenolol, prenalterol, adiphenine, prednisolone diethylaminoacetate, or 25 reserpine.

57. Combination according to any of preceding claims, wherein the said at least one third or second amphipatic substance takes the role of a drug as the as the carboxylic acid salt or the carboxylic acid 30 compound that can be converted into the carboxylic acid salt by salt

- 149 -

formation, methylprednisolone sodium succinate, prednisolone sodium succinate, 3,20-dioxo-5 β -pregnane, hydroxydione succinate sodium, 11,20-dioxo-3 α -hydroxy- 5 α -pregnane, alphadolon, a cholic acid or deoxycholic acid salt, alclofenac, ibufenac, ibuprofen, clindanac, fenclo rac,

5 ketoprofen, fenoprofen, indoprofen, fenclofenac, diclofenac, flurbiprofen,

pirprofen, naproxan, benoxaprofen, carprofen, cicloprofen, mefenamic acid, flufenamic acid, tolfenamic acid, meclofenamic acid, milflumic acid, clonixin, flunixin, indometacin, oxmetacin, intrazol, acemetazin, cinmetacin, zomepirac, tolmetin, colpirac, tiaprofenic acid, benzadac, PGE2

10 (dinoprostone), PGF2 α (dinoprost), 15 (S)-15-methyl-PGE2, 15 (S)-15-methyl-PGF2 α (carboprost), (\pm)15 (Xi)-15-methyl-13,14-dihydro-11-deoxy-PGE1 (deprostil), 15 (S)-15-methyl-11-deoxy-PGE, (doxaprost), 16,16-dimethyl-PGE2, 17-phenyl-18,19,20-trinor-PGF2 α , 16-phenoxy-17,18,19,20-tetranor-PGF2, or N-methylsulphonyl-16-phenoxy-17,18,19,20-

15 tetranor-PGF2 α (sulproston), nalixidic acid, cinoxacin, oxolinic acid,

pironidic acid, piperidic acid, penicillin G or V, phenethicillin, propicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, flucloxacillin, cyclacillin, epicillin, mecillinam, methicillin, azlocillin, sulbenicillin, ticarcillin, mezlocillin,

20 piperacillin, carindacillin, azidocillin, ciclazillin, cefaclor, cefuroxime, cefazlur,

cepha cetrile, cefazolin, cepha lexin, cefadroxil, cepha loglycin, cefoxitin, cepha loridine, cephsulodin, cefotiam, ceftazidine, cefonicid, cefotaxime, cefmenoxime, ceftizoxime, cephalothin, cephradine, cefamandol, cephanone, cephapirin, cefroxadin, cefatrizine, cefazedonep ceftrixon, ceforanid, moxalactam, clavulanic acid, nocardicine A, sulbactam, aztreonam,

25 thienamycin, chlorambucil or .methotrexate.

58. Combination according to any of preceding claims, wherein the said at least one third or second amphipatic substance, which takes to role of a drug, acts as an adrenocorticostatic , a β -adrenolytic, an

30 androgen an antiandrogen, an antiparasitic, an anabolic, an anaesthetic, an

- 150 -

analgesic, an analeptic, an antiallergic, an antiarrhythmic, an
antiarterosclerotic, an antiasthmatic, a bronchospasmolytic, an antibiotic, an
antidrepressive, an antipsychotic, an antidiabetic, an antidot, an antiemetic,
an antiepileptic, an antifibrinolytic, an anticonvulsive, an anticholinergic, an
5 enzyme, a coenzyme or corresponding inhibitor, an antihistaminic, an
antihypertonic, a biological inhibitor of drug activity, an antihypotonic, an
anticoagulant, an antimycotic, an antimyasthenic, an agent against Morbus
Parkinson or Morbus Alzheimer, an antiphlogistic, an antipyretic, an
antirheumatic, an antiseptic, a respiratory analeptic or a respiratory stimulant,
10 a broncholytic, a cardiotonic, a chemotherapeutic, a coronary dilatator, a
cytostatic, a diuretic, a ganglium-blocker, a glucocorticoid, an antiflow agent,
a haemostatic, a hypnotic, an immunoglobuline or its fragment, an
immunologically active substance, a bioactive carbohydrate, a bioactive
carbohydrate derivative, a contraceptive, an anti-migraine agent, a mineralo-
15 corticoid, a morphine-antagonist, a muscle relaxant, a narcotic, a
neurotherapeutic, a neuroleptic, a neurotransmitter or its antagonist, a small
peptide, a small peptide derivative, an ophthalmic, a sympaticomimetic or a
sympathicolytic, a para-sympaticomimetic or a para-sympathicolytic, a
psoriasis drug, a neurodermitis drug, a mydriatic, a psychostimulant, a
20 rhinologic, a sleep-inducing agent or its antagonist, a sedating agent, a
spasmolytic, tuberculostatic, an urologic agent, a vasoconstrictor or
vasodilatator, a virustatic, a wound-healing substance, or a combination of
aforesaid agents.

25 59. Combination according to any of preceding claims,
wherein the drug content is between 0.1 rel.% and 60 rel.% compared to the
total mass of all three said substances that form said extended surfaces.

60. Combination according to any of preceding claims,
wherein said at least one third or second substance is a low molecular weight
immunomodulator.

5 61. Combination according to any of preceding claims,
wherein said at least one third or second substance is a bio-catalyst.

62. Combination according to any of preceding claims,
wherein said at least one third or second substance is a low molecular weight
10 agonist or antagonist of some biological substance action.

63. Combination according to any of preceding claims,
wherein said at least one third or second substance is a co-enzyme.

15 64. Combination according to any of preceding claims,
wherein said at least one third or second substance is a hormone.

65. Combination according to any of preceding claims,
wherein said at least one third or second substance is a low to intermediate
20 weight polypeptide with membrane destabilising properties.

66. Combination according to any of preceding claims,
wherein said at least one second substance is a cyclooxygenase or
lipoxigenase inhibitor and at least one third substance is a non-ionic
25 surfactant with solubility in 1-10 μ M range that preferably belongs to the class
of sorbitane-polyoxyethylene-alkyl or -alkylene esters or else is a
polyoxyethylene-alkyl or -alkylene ether.

67. The use of a combination of substances according to any of
30 preceding claims, in drug carriers, drug depots, or for other kind of medicinal

or biological application by providing the extended surfaces in the form of membranes formed by the at least one first substance, the at least one second and the at least one third substance, which together surround miniature droplets, wherein the substance with biological activity, being a drug, is mainly associated with said droplet surface or else is mainly incorporated into the droplet and then carried by the droplet to the place where the drug is intended to act.

68. The use of a combination of substances according to any of preceding claims for the manufacture of a preparation for the transport of an active ingredient, which can be one of the three amphipatic components, especially for biological, medical, immunological, or cosmetic purposes, into and through the skin of warm blood creatures.

69. A method of preparing a combination according to any of preceding claims in the form of a formulation of a biologically, cosmetically and/or pharmaceutically active agent, comprising the steps of

- selecting the at least one first and the at least one second substance which together form extended surfaces, when in contact with said medium, such that said extended surfaces formed by the at least one first and the at least one second substance are more adaptable than the at least one first substance alone and the surfaces formed by the at least one second substance alone form small aggregates; alternatively
- selecting the at least one first and the at least one third substance which together form extended surfaces, when in contact with said medium, such that said extended surfaces formed by the at least one first and the at least one third substance are more adaptable than the at least one first substance alone and the surfaces formed by the at least one third substance alone form small aggregates, if this substance self-aggregates; and

- 153 -

- generating said surface-forming combination from at least one first, at least one second, and at least one third substance, such that the surface of resulting at least three component combination is even more adaptable than the surface prepared from at least one first and one second substance alone or of the surfaces formed by the at least one first and one third substance alone, bringing the combination of at least two or all three said substances into suspension by means of controlled mechanical fragmentation, in the presence of or before being mixed with the at least one third substance, such that said third substance is incorporated at least partly in said extended surface formed by controlled mechanical fragmentation to obtain final preparation.

70. The method according to any of preceding claims, wherein said means of controlled mechanical fragmentation includes on filtration, pressure change or mechanical homogenisation, shaking, stirring, or mixing.

71. The method according to any of preceding claims, wherein the liquid medium suspension characteristics correspond to any one of claims 1 to 65.

72. The method according to any of preceding claims, wherein said active agent is selected from the group comprising anti-diabetic agents, growth factors, immunomodulators, enzymes, recognition molecules, adrenocorticostatics, adrenolytics, androgens, antiandrogens, antiparasitics, anabolics, anaesthetics, analgesics, analeptics, antiallergics, antiarrhythmics, antiarterosclerotics, antiasthmatics, bronchospasmolytics, antibiotics, antidrepressiva, antipsychotics, antidots, antiemetics, antiepileptics, antifibrinolytics, anticonvulsiva, anticholinergics, enzyme, coenzymes or corresponding inhibitors, antihistaminics, antihypertonics, biological inhibitors

- 154 -

of drug activity, antihypotonics, anticoagulants, antimycotics,
antimyasthenics, agents against Morbus Parkinson or Morbus Alzheimer,
antiphlogistics, antipyretics, antirheumatics, antiseptics, respiratory
analeptics or respiratory stimulants, broncholytics, cardiotonics,
5 chemotherapeutics, coronary dilators, cytostatics, diuretics, ganglium-
blockers, glucocorticoids, antiflow agents, haemostatics, hypnotics,
immunologically active substances, contraceptives, anti-migraine agents,
mineralo-corticoids, morphine-antagonists, muscle relaxants, narcotics,
neurotherapeutics, neuroleptics, neurotransmitters or their antagonists,
10 peptides, peptide derivatives, ophthalmics, sympaticomimetics or
sympathicolitics, para-sympaticomimetics or para-sympathicolitics, anti-
psoriasis drugs, neurodermitis drugs, mydriatics, psychostimulants,
rhinologics, sleep-inducing agents or their antagonists, sedating agents,
spasmolytics, tuberculostatics, urologics, vasoconstrictors or vasodilators,
15 virustatics, wound-healing substances, or a combination of aforesaid agents.

73. The method according to any of preceding claims,
wherein said at least three amphiphilic substances are either used as such,
or dissolved in a physiologically compatible polar fluid, comprising water or
20 water-miscible fluids, or in a solvation-mediating agent, together with a polar
solution.

74. The method according to any of preceding claims,
wherein the said polar solution contains at least one surfactant or surfactant-
25 like amphipat, which destabilises bilayer membrane, and at least one more
membrane destabilising, biologically active ingredient or an additional
surfactant.

75. The method according to any of preceding claims, wherein the formation of said surfaces is induced by substance addition into a fluid phase, evaporation from a reverse phase, by injection or dialysis, or with the aid of mechanical stress.

5

76. The method according to any of preceding claims, wherein the formation of said surfaces is induced by filtration, the filtering material having pores diameters between 0.01 μm and 0.8 μm , the preferred choice of pore diameter being dependent on the desired final aggregate dimensions.

10

77. The method according to any of preceding claims, wherein several filters are used sequentially or in parallel.

15

78. The method according to any of preceding claims, wherein said agents and carriers are made to associate, at least partly, after formation of said extended surfaces.

20

79. The method according to any of preceding claims, wherein said extended surfaces, with which the agent molecules are allowed to associate, are prepared just before the application of the formulation, if convenient from a suitable concentrate or a lyophilisate.

25

80. A container comprising the pharmaceutical composition based a combination of substances according to any preceding claim.

30

81. A package comprising at least one container comprising the pharmaceutical composition based on a combination of substances according to any preceding claims.

82. A method for generating a therapeutic effect on a warm blood creature by applying a pharmaceutical composition based on a combination of substances according to any of previous claims onto or into such living creature body.

5

83. The method according to any of preceding claims, wherein different administration volumes are selected to control the applied medicament dose and the outcome of therapeutic application.

10

84. The method according to any of preceding claims, wherein a suspension of drug-free aggregates is loaded with the drug to be associated therewith during the day prior to an administration, preferably 360 min, more preferably 60 min and even more preferably 30 min before administering the resulting formulation in or on the body.

15

85. The method of any of preceding claims, characterised in that at least one dose of the pharmaceutical composition with therapeutic activity is administered.

20

86. The method according to any of preceding claims, wherein the flux of penetrants that carry a drug through the various pores in a well-defined barrier is determined as a function of a suitable driving force or a pressure acting across the barrier and the data are then conveniently described by a characteristic curve which, in turn, is employed to optimise the formulation or application further.

25

87. The method according to any of preceding claims, wherein the characteristic, e.g. penetrability vs. pressure, curve is analysed in terms of eq. (*) or alike.

30

88. The combination of any of preceding claims, wherein the adaptability of extended surface aggregates comprising all three said amphipatic components exceeds by at least 20% or by at least twice the standard deviation of a typical measurement, whichever is smaller, the
5 adaptability of the extended surface aggregates comprising the at least one first and the at least one second amphipatic component, used at the corresponding concentrations, or the adaptability of the extended surface comprising the at least one first and the at least one third amphipatic component, used at the corresponding concentrations, whichever is smaller.

10

89. The combination of any of preceding claims, wherein the adaptability of extended surface aggregates comprising all three said amphipatic components exceeds by at least 30% the adaptability of the extended surface aggregates comprising the at least one first and the at least
15 one second amphipatic component, used at the corresponding concentrations, or the adaptability of the extended surface comprising the at least one first and the at least one third amphipatic component, used at the corresponding concentrations, whichever is smaller.

20

90. The combination of any of preceding claims, wherein the total concentration of said at least one second and said at least one third compound in the ESAs comprising all three said amphipatic components is equal to or less than the concentration of said at least one second compound in the ESAs comprising at least one first and at least one second compound
25 and the corresponding concentration of the at least one first compound.

91. The combination of any of preceding claims, wherein the total concentration of said at least one second and said at least one third compound in the ESAs comprising all three said amphipatic components is
30 equal to or less than the concentration of said at least one third compound in

the ESAs comprising at least one first and at least one second compound at the corresponding concentration of the at least one first compound.

92. The combination of any of preceding claims, wherein the adaptability is
5 expressed as the inverse value of the p^* value corresponding to a predefined fraction of P_{max} -value, which is often selected around 60% and preferably is 57% of P_{max} -value.

93. Use of an additional at least third amphipatic component as at least
10 second membrane destabilizing compound to increase the adaptability of ESAs that otherwise would comprise only two amphipatic compounds, one of which is membrane forming and one of which is the first membrane destabilising compound, to obtain the corresponding three-component ESAs, comprising one membrane forming and two membrane destabilising
15 compounds, such that the latter kind of ESAs have higher adaptability than the former ESAs.

94. Use of the third amphipatic component according to claim 93 in a combination according to any of preceeding claims 1 to 93.
20

95. A suspension of extended surface aggregates in a liquid medium comprising:
at least one first amphipatic component;
at least one second amphipatic component;
25 at least one third amphipatic component;
the first amphipatic component being a membrane forming lipid component;
the second and third component being membrane destabilising components;
wherein the third component is a non-steroidal anti-inflammatory drug (NSAID);
and

whereby the aggregates are capable of penetrating semi-permeable barriers with pores at least 50% smaller than the average aggregate diameter before the penetration without changing the aggregate diameter by more than 25%.

5

96. A suspension of extended surface aggregates in a liquid medium comprising:

at least one first amphipatic component;

at least one second amphipatic component;

10

at least one third amphipatic component;

the first amphipatic component being a membrane forming lipid component;

the second and third component being membrane destabilising components;

wherein the third component is a NSAID; and

15

whereby extended surface aggregate comprising the first and second (but not the third) component, or the first and third (but not the second) component, the

second or third component being present at a relative concentration X with respect to the concentration of the first component, have a lower propensity to overcome barriers with pores at least 50% smaller than the average aggregate diameter before the pore crossing than the extended surface aggregates

20

comprising the first, second and third component together, whereby the concentration of the combined second and third components is at or below the relative concentration X.

25

97. The suspension according to claims 95 or 96, said extended surface aggregates being membrane-enclosed, liquid-filled vesicles, said first component being a membrane-forming lipid, and said second and third components being membrane-destabilising components.

30

98. A suspension of extended surface aggregates in a liquid medium comprising:

- at least one first amphipatic component;
- at least one second amphipatic component;
- at least one third amphipatic component;
- the first amphipatic component being a membrane forming lipid component;
- 5 the second and third component being membrane destabilising components;
- wherein the third component is a non-steroidal anti-inflammatory drug (NSAID);
- and

whereby the extended surface aggregates are capable of penetrating intact mammalian skin, thus increasing NSAID concentration in the skin and/or

10 increasing the reach of NSAID distribution beyond the skin, in comparison with the result of the same NSAID application in a solution on the skin.

99. The suspension of claim 98, said extended surface aggregates being membrane-enclosed, liquid-filled vesicles, said first component being a

15 membrane-forming lipid, and said second and third components being membrane-destabilising components.

100. The suspension of any preceeding claim, wherein the third (NSAID) component is ketoprofen, ibuprofen, diclofenac, indomethacin, naproxen or

20 piroxicam.

101. The suspension of any preceeding claim, wherein the first component is selected from the group consisting of phosphatidylcholines,

phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols,

25 phosphatidic acids, phosphatidylserines, sphingomyelins, sphingophospholipids, glycosphingolipids, cerebrosides, ceramidpolyhexosides, sulphatides, sphingoplasmalogenes, or gangliosides.

102. The suspension of claim 101, wherein the first component is a

30 phosphatidylcholine of biological, preferably plant, origin, especially soy (bean),

coconut, olive, safflower or sunflower, linseed, evening primrose, primrose, or castor oil, etc..

103. The suspension of any preceding claim, wherein the second component
5 is a surfactant.

104. The suspension of claim 103, wherein the surfactant has a solubility in the liquid medium ranging from about 5×10^{-7} M to about 10^{-2} M.

105. The suspension of claim 103, wherein the surfactant has hydrophilicity-lipophilicity ratio (HLB) between 10 and 20, even better between 12 and 18 and most preferred between 13 and 17.

106. The suspension of claim 102, wherein the surfactant is selected from
15 the group of nonionic surfactants, and preferably is a polyethyleneglycol-sorbitan-long fatty chain ester, from polyethyleneglycol-long fatty chain ester or -ether, a polyhydroxyethylen-long fatty chain ester or -ether, or a surfactant-like nonionic phospholipid.

107. The suspension of any preceding claim, wherein the first component is
20 a phosphatidylcholine and the third (NSAID), component is ketoprofen, diclofenac, ibuprofen, indomethacin, naproxen, or piroxicam.

108. The suspension of claim 13, wherein the second component is a non-
25 ionic surfactant, preferably is a polyethyleneglycol-sorbitan-long fatty chain ester, polyethyleneglycol-long fatty chain ester or polyethyleneglycol-long fatty chain ether type, the polyethyleneglycol chain being potentially replaced by a polyhydroxyethylene polar group, or else is a surfactant-like nonionic phospholipid.

109. The suspension of any preceding claim, wherein the average aggregate diameter before the aggregates penetrate the pores is at least 40% larger than the average pore diameter.

5 110. The suspension of any preceding claim, wherein the first component and the second component differ in solubility in the liquid medium at least 10-fold, on the average.

10 111. The suspension any preceding claim, wherein the second component and the third component differ in solubility on the average at least 2-fold.

112. The suspension of any preceding claim, wherein the total dry mass of the at least three amphipatic components is between 0.01 weight-% and 50 weight-%.

15 113. The suspension according to any preceding claim, wherein the extended surfaces formed by the at least three components have an average curvature corresponding to an average diameter between 15 nm and 5000 nm.

20 114. The suspension according to any previous claim, wherein the at least one further membrane destabilising component is a lower aliphatic alcohol.

115. A pharmaceutical preparation comprising the suspension of claims 95 to 114.

25 116. A pharmaceutical preparation comprising a suspension of liquid-filled vesicles in an aqueous medium, the vesicles being enclosed by membranes formed from at least one lipid component and comprising at least two membrane destabilising components,

whereby extended surface aggregate comprising the first and second (but not the third) component, or the first and third (but not the second) component, the second or third component being present at a relative concentration X with respect to the concentration of the first component, have a lower propensity to overcome barriers with pores at least 50% smaller than the average aggregate diameter before the pore crossing than the extended surface aggregates comprising the first, second and third component together, whereby the concentration of the combined second and third components is at or below the relative concentration X.

10

117. A pharmaceutical preparation comprising a suspension of liquid-filled vesicles in an aqueous medium, the vesicles being enclosed by membranes formed from at least one lipid component and comprising at least three membrane destabilising components, whereby the membrane destabilising components comprise a surfactant, a non-steroidal anti-inflammatory drug, and / or a lower aliphatic alcohol, whereby the membrane destabilising components increase the vesicle ability to penetrate mammalian skin and thus increase the reach of NSAID distribution in the skin, and beyond, in comparison with the result of an NSAID application in a solution on the skin.

20

118. The pharmaceutical preparation of claim 117, wherein the first component is phosphatidylcholine and the third component is an NSAID, such as ketoprofen, diclofenac, ibuprofen, indomethacin, naproxen, or piroxicam.

25

119. The pharmaceutical preparation of claim 117, wherein the surfactant is selected from the group of nonionic surfactants, and preferably is a polyethyleneglycol-sorbitan-long fatty chain ester, a polyethyleneglycol-long fatty chain ester or a polyethyleneglycol-long fatty chain ether, the polyethyleneglycol chain being potentially replaced by a polyhydroxyethylene polar group, or else is a nonionic, surfactant like phospholipid.

30

120. The pharmaceutical preparation of claim 117, wherein the alcohol is n-propanol, iso-propanol, 2-propanol, n-butanol, 2-butanol, 1,2-propanediol, 1,2-butanediol, or ethanol.

5

121. The pharmaceutical preparation of claim 117, wherein the bulk pH value is above the logarithm of the apparent dissociation constant (pKa) of NSAID drug in a solution and in extended surface aggregates, and the latter pKa is higher than the former.

10

122. The pharmaceutical preparation of claim 117, wherein the bulk pH value is between 6.4 and 8.3, more preferably is between 6.7 and 8 and most preferably is between 7 and 7.7.

15

123. The pharmaceutical preparation of claim 117, wherein the bulk ionic strength is between 0.005 and 0.3, even better is between 0.01 and 0.2 and best is between 0.05 and 0.15.

20

124. The pharmaceutical preparation of claim 117, wherein the formulation viscosity is between 50 mPa s and 30.000 mPa s, preferably is between 100 mPa s and 10.000 mPa s, more preferably is between 200 mPa s and 5000 mPa s, and most preferred is between 400 mPa s and 2000 mPa s.

25

125. The pharmaceutical preparation of claim 117, wherein the first, i.e. phospholipid, component and the third, i. e. NSAID, component are present in the suspension in a relative molar ratio between 10/1 and 1/1.

30

126. The pharmaceutical preparation of claims 117, wherein the first, i.e. a phospholipid, component, and the second, i.e. a surfactant, component, are present in the suspension in a relative molar ratio between 40/1 and 4/1.

127.A kit comprising, in a tube or otherwise packaged form, at least one dose of the pharmaceutical preparation according to any one of claims 115 to 126.

5

128.A method for treating peripheral pain and/or inflammation by applying a pharmaceutical preparation according to any one of claims 114 to 127 on the skin of a warm blooded mammal.

10 129.The method according to claim 128 wherein different formulation doses per area are selected to control the depth of drug delivery.

130.The method according to 127 and 128. wherein the pharmaceutical formulation is applied in a non-occlusive patch.

15

131.Use of transdermal carriers according to any preceding claim to deliver NSAID molecules below the skin and into the subcutaneous muscle, and/or subcutaneous joint.

1/8

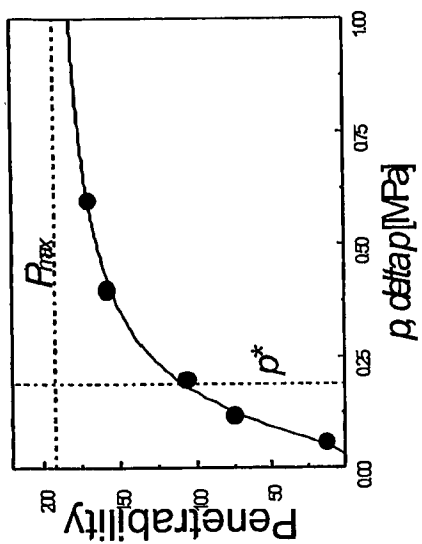


Fig. 3

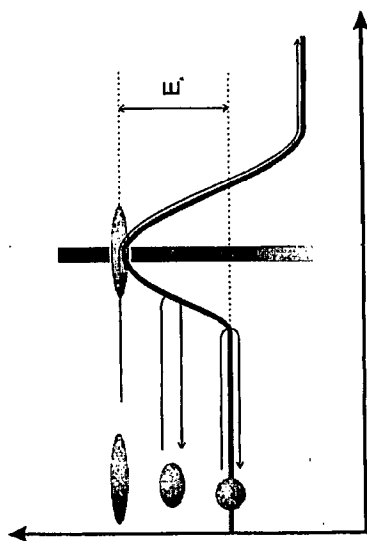


Fig. 2

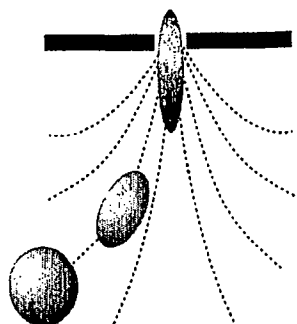


Fig. 1

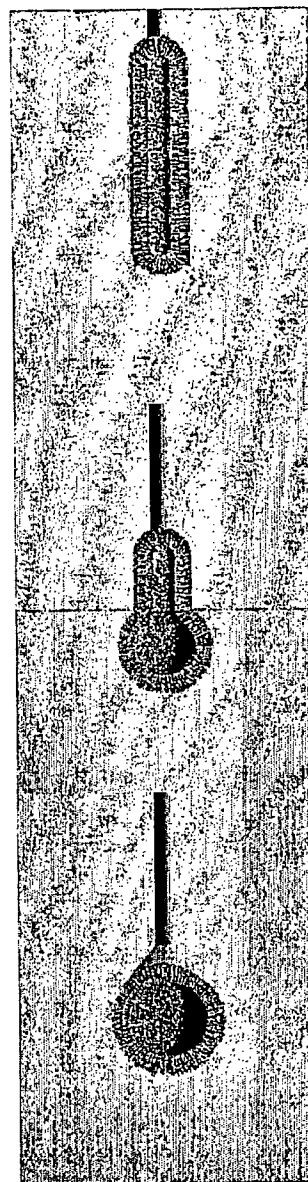


Fig. 4

2/8

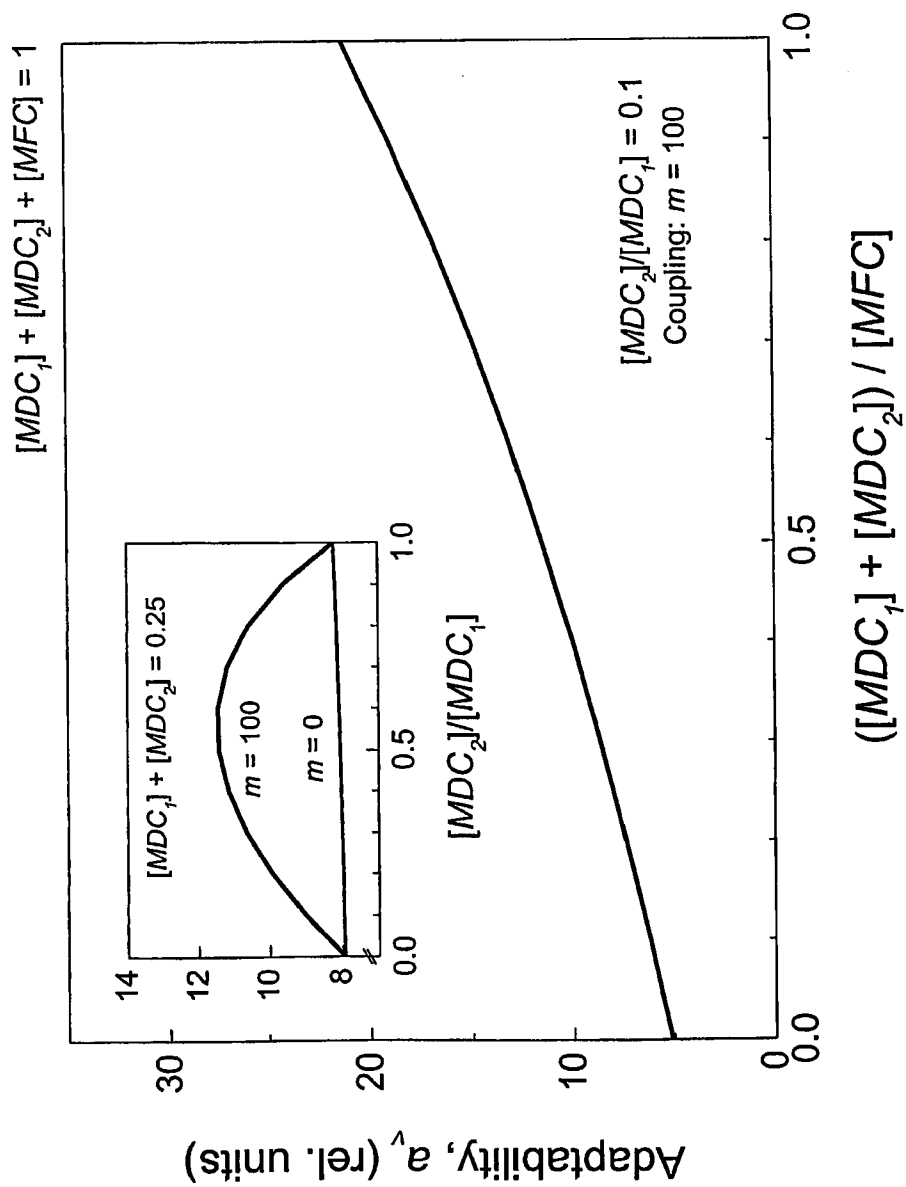
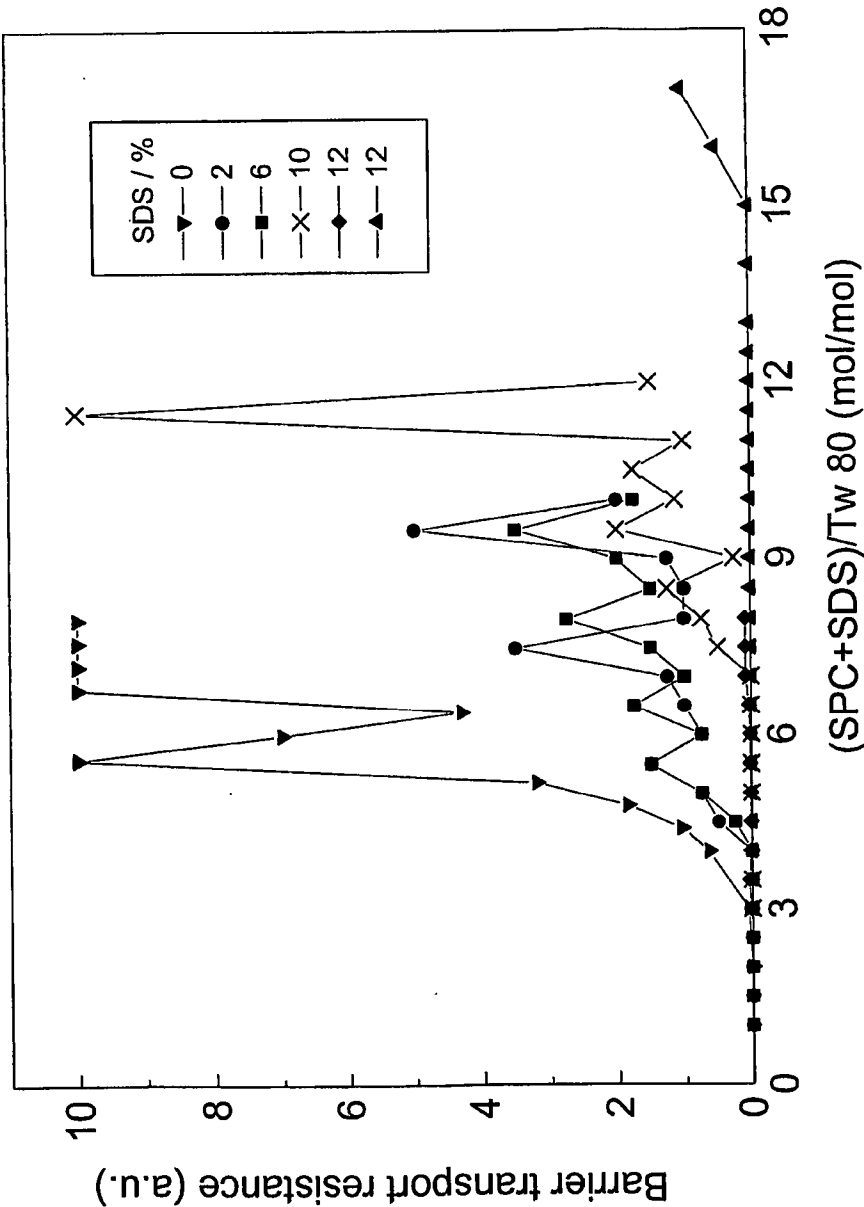


Fig. 5

Fig. 6



4/8

Fig. 7

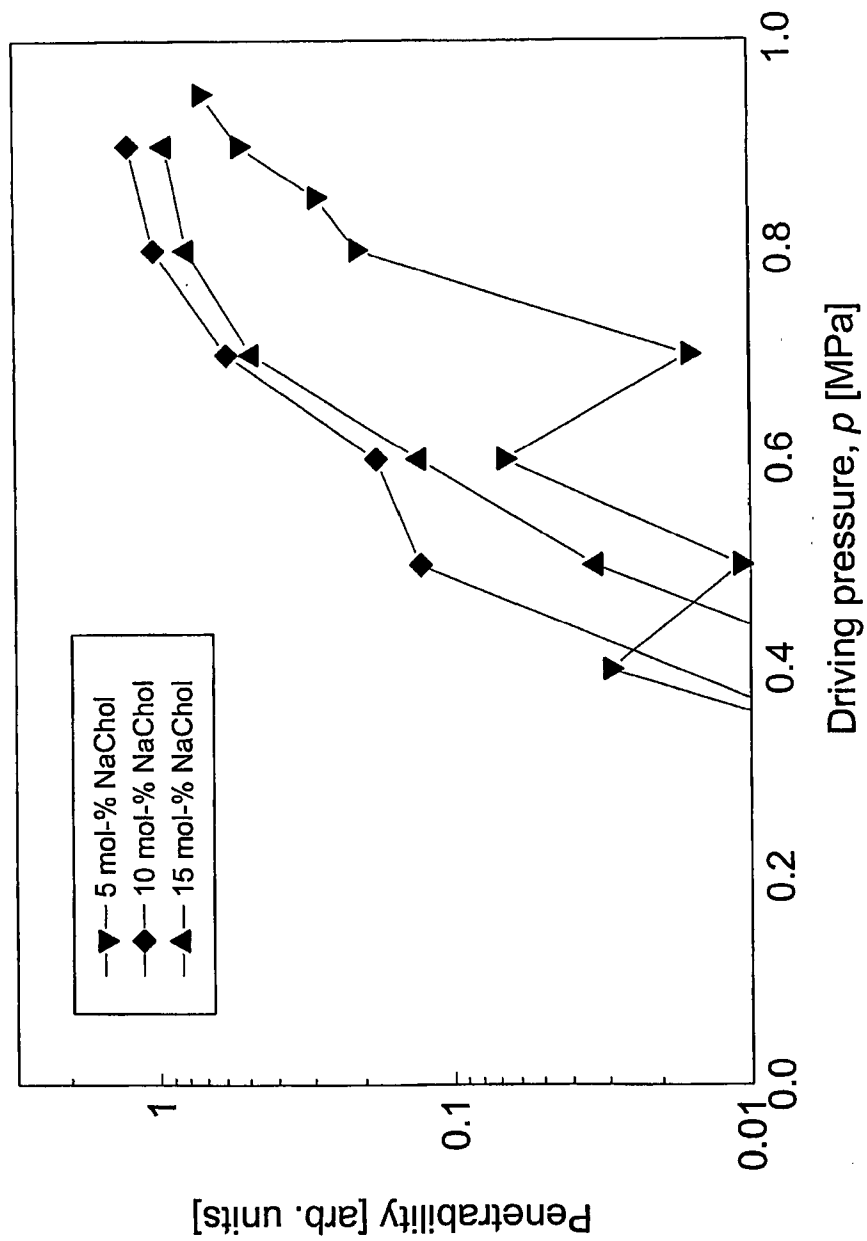


Fig. 8

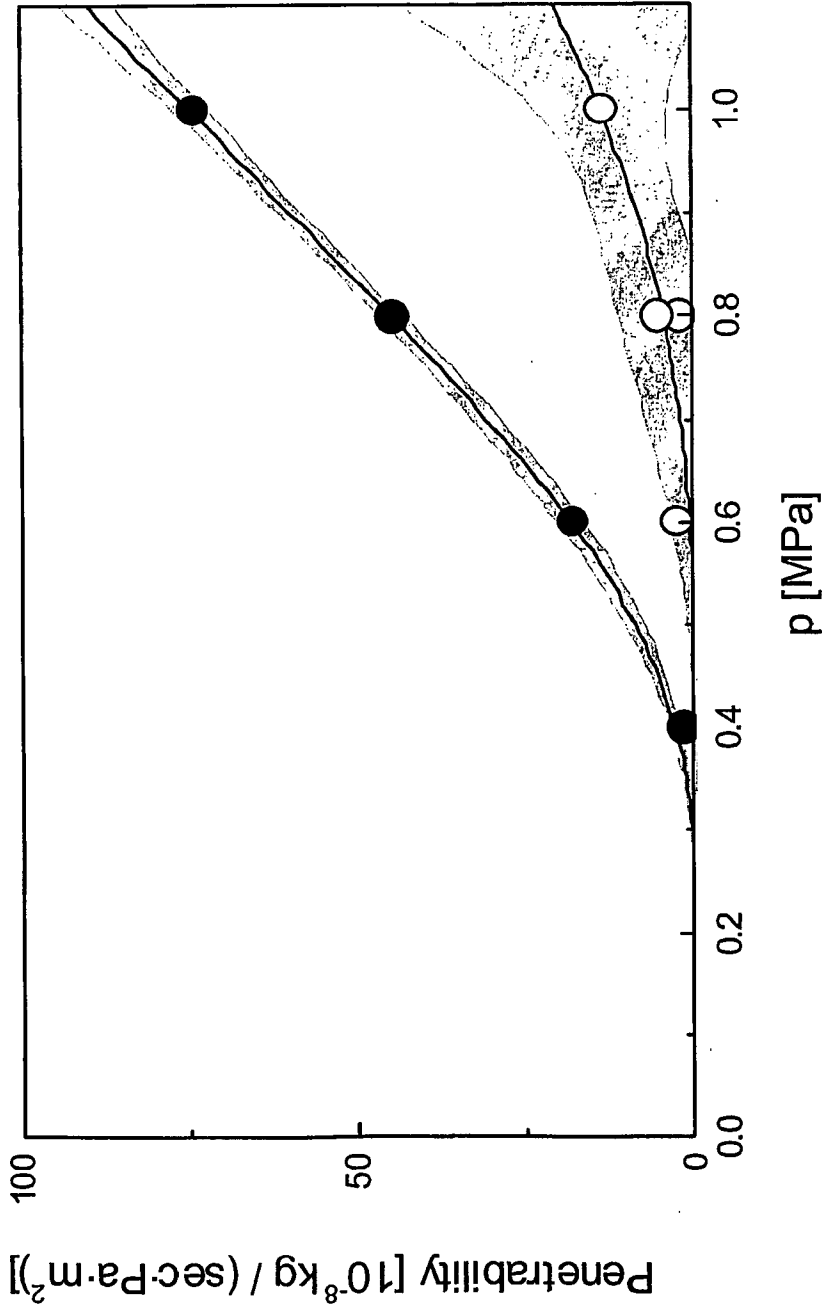
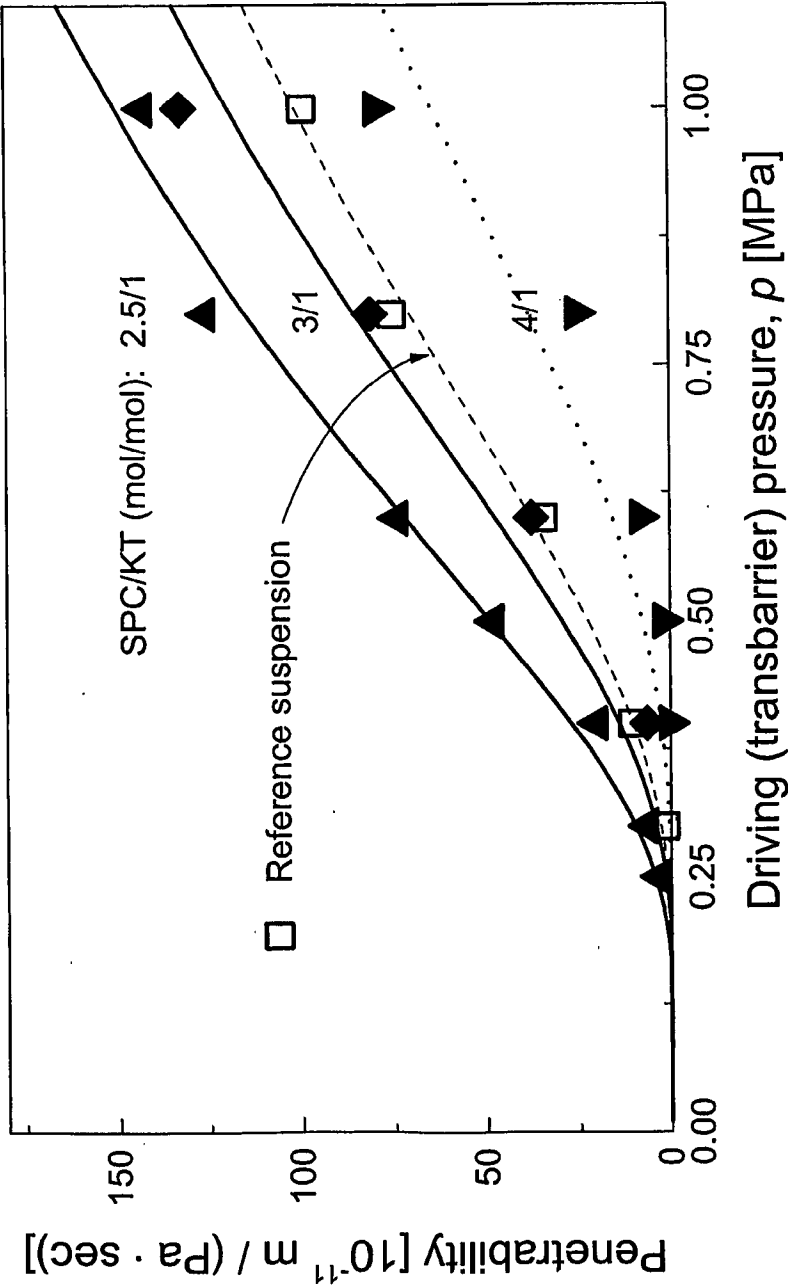


Fig. 9



7/8

Fig. 10

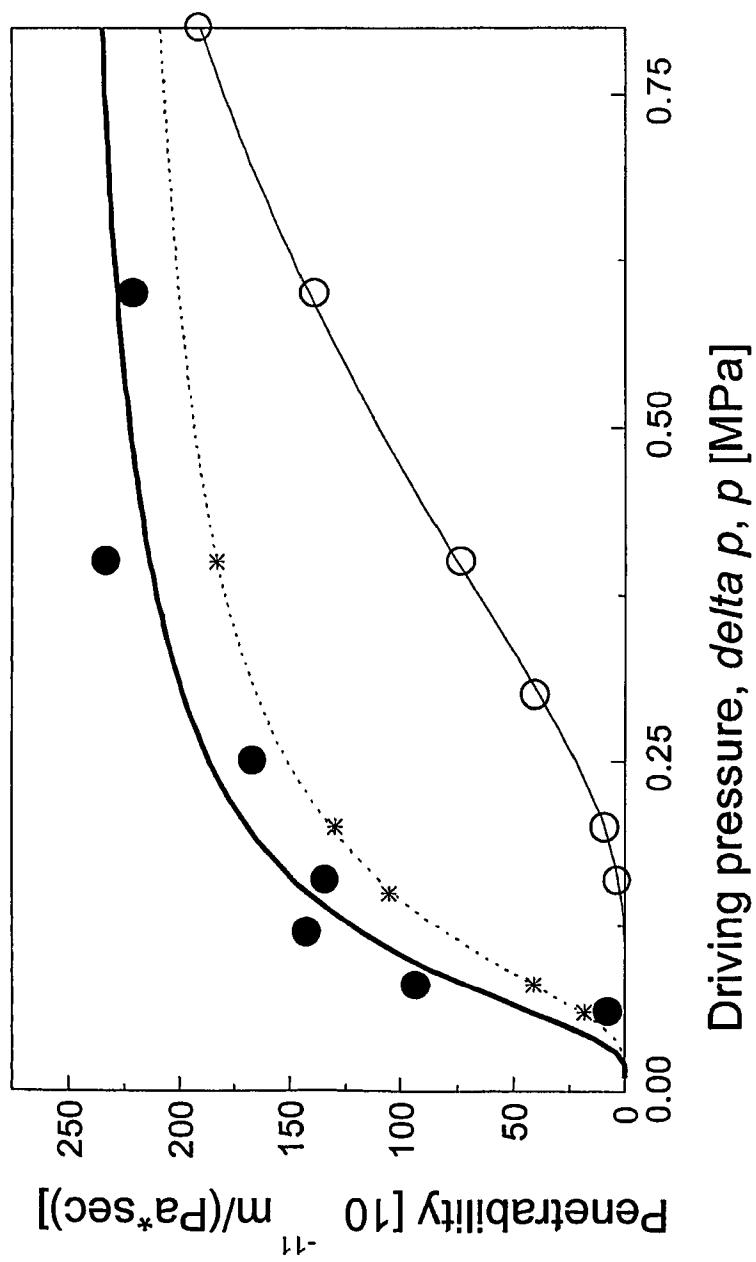
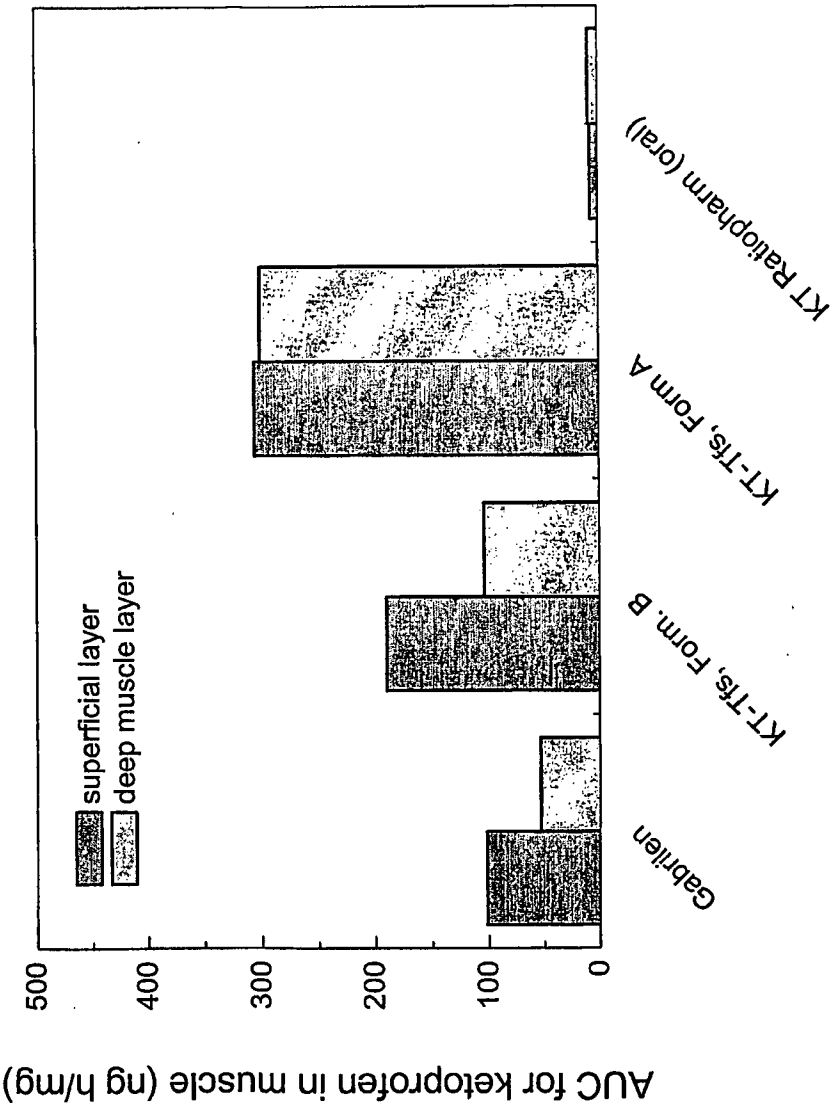


Fig. 11



INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 03/11202

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K9/127 A61K31/195

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, MEDLINE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>CEVC G ET AL: "ULTRAFLEXIBLE VESICLES, TRANSFERSOMES, HAVE AN EXTREMELY LOW PORE PENETRATION RESISTANCE AND TRANSPORT THERAPEUTIC AMOUNTS OF INSULIN ACROSS THE INTACT MAMMALIAN SKIN"</p> <p>BIOCHIMICA ET BIOPHYSICA ACTA: BIOMEMBRANES, AMSTERDAM, NL, vol. 1368, no. 2, 19 January 1998 (1998-01-19), pages 201-215, XP001119925 ISSN: 0005-2736 abstract page 202, paragraph 2.4 page 203, paragraph 2.5 figures 2-5</p> <p style="text-align: center;">--- -/-</p>	1-131



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the International filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principles or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the International search

21 January 2004

Date of mailing of the international search report

30/01/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Villa Riva, A

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 03/11202

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 475 160 A (CEVC GREGOR) 18 March 1992 (1992-03-18) the whole document, in particular page 17, line 16 - line 29 examples ---	1-131
X	EP 1 031 347 A (IDEA AG) 30 August 2000 (2000-08-30) the whole document, in particular paragraphs '0160!-'0163! examples ---	1-131
X	DE 44 47 287 C (CEVC GREGOR) 7 November 1996 (1996-11-07) pages 5-7, sections "Lipide" and "Wirkstoffe", in particular page 7, line 3-5 examples ---	1-131
X	DE 41 07 153 A (CEVC GREGOR) 10 September 1992 (1992-09-10) the whole document ---	1-131
A	CEVC G: "Transfersomes, liposomes and other lipid suspensions on the skin: permeation enhancement, vesicle penetration, and transdermal drug delivery" CRITICAL REVIEWS IN THERAPEUTIC DRUG CARRIER SYSTEMS, XX, XX, vol. 13, no. 3/4, 1996, pages 257-388, XP002107366 ISSN: 0743-4863 the whole document -----	1-131

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-131 (partially)

Present claims 1-131 relate to a compositions, methods and uses as drug carriers of a combination of amphiphiles defined by reference to several desirable properties, e.g. ability to form and destabilize membranes, produce particles with certain size, having certain relative solubilities etc.

The claims cover all compositions, methods and uses relating to combinations of compounds having these characteristics or properties, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such combinations. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). Moreover, the present claims relate to an extremely large number of possible drugs which can be included into the aggregates, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such active principles, namely some NSAIDS.

Therefore, the search has been limited to the examples and to preparations with at least three amphiphiles, of which one can be the active principle, and provided with high deformability, and their uses in medicine.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/EP 03/11202

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 82-87, 128-130 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 1-131 (partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 03/11202

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0475160	A	18-03-1992	DE 4107152 A1 10-09-1992
			DE 4107153 A1 10-09-1992
			AT 134133 T 15-02-1996
			CA 2067754 A1 25-02-1992
			DE 59107402 D1 28-03-1996
			DK 475160 T3 08-07-1996
			WO 9203122 A1 05-03-1992
			EP 0475160 A1 18-03-1992
			ES 2085936 T3 16-06-1996
			JP 5502042 T 15-04-1993
			US 6165500 A 26-12-2000
EP 1031347	A	30-08-2000	EP 1031347 A1 30-08-2000
			AT 216223 T 15-05-2002
			AU 2293400 A 18-08-2000
			BR 0007780 A 05-02-2002
			CA 2360643 A1 03-08-2000
			CN 1344155 T 10-04-2002
			DE 69901284 D1 23-05-2002
			DE 69901284 T2 28-11-2002
			DK 1031347 T3 08-07-2002
			WO 0044350 A1 03-08-2000
			EP 1143932 A1 17-10-2001
			ES 2173679 T3 16-10-2002
			HK 1030364 A1 22-11-2002
			HU 0105400 A2 29-05-2002
			JP 2002535351 T 22-10-2002
			PT 1031347 T 30-09-2002
			SI 1031347 T1 31-10-2002
DE 4447287	C	07-11-1996	CN 1234736 A 10-11-1999
			WO 9817255 A1 30-04-1998
			DE 4447287 C1 07-11-1996
			AU 724218 B2 14-09-2000
			AU 4510897 A 15-05-1998
			BR 9612750 A 28-12-1999
			EP 0935457 A1 18-08-1999
			HU 9903363 A2 28-03-2000
			JP 2001502676 T 27-02-2001
			KR 2000049266 A 25-07-2000
			US 2002048596 A1 25-04-2002
DE 4107153	A	10-09-1992	CA 2268670 A1 30-04-1998
			DE 4107153 A1 10-09-1992
			AT 134133 T 15-02-1996
			CA 2067754 A1 25-02-1992
			DE 59107402 D1 28-03-1996
			DK 475160 T3 08-07-1996
			WO 9203122 A1 05-03-1992
			EP 0475160 A1 18-03-1992
			ES 2085936 T3 16-06-1996
			JP 5502042 T 15-04-1993
			US 6165500 A 26-12-2000